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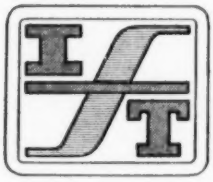

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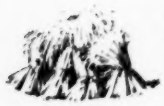
The Vital Story of
MACARONI* ENRICHMENT

by Science Writer

This is the fourth article in a series devoted to the story of cereal enrichment



Dramatic results have been recorded about the value of enrichment in improving health. From the United States, the Bataan peninsula in the Philippines, Newfoundland and many other parts of the world comes word of the great benefits which result from enrichment.

For years, some forward-looking manufacturers of macaroni and noodle products have used enrichment to make their good foods better. They know that enrichment restores important vitamin and mineral values which are unavoidably lost in milling, and they recognize their responsibility to provide the greatest health-building benefits for the public.

Enrichment is really a simple process. It adds the following essential elements to the food during manufacture.

Thiamine—also called vitamin B_1. This vitamin helps to build physical and mental health. It is essential for normal appetite, intestinal activity and sound nerves.

Riboflavin—also called vitamin B_2. This vitamin helps to keep body tissues healthy and to maintain proper function of the eyes. It is essential for growth.

Niacin—another "B" vitamin, is needed for healthy body tissues. Its use in the American diet has done much to make a serious disease called pellagra disappear.

Iron—is a mineral used in all enrichment. It is essential for making good, red blood and preventing nutritional anemia.

Products made from semolina may be enriched by two methods. One uses small square wafers which contain all the vitamins and iron necessary to enrich 100 lbs. The wafers break up in a small amount of water which is then added to the paste. For manufacturers who use the continuous press method, a powdered concentrate of the vitamins and iron, called a premix, is available. This is added by a mechanical feeding device.

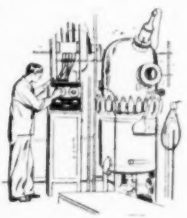
**Macaroni is used here in the generic sense. It includes all alimentary pastes: macaroni, spaghetti, pastina, noodles.*

These are the minimum and maximum levels, in milligrams per pound, required by the Federal Definitions and Standards of Identity for enriched alimentary pastes.

	Min.	*Max.*
Thiamine (vitamin B_1)	4.0	5.0
Riboflavin (vitamin B_2)	1.7	2.2
Niacin	27.0	34.0
Iron	13.0	16.5

NOTE: These levels allow for 30% to 50% losses in kitchen procedures.

Nowadays scientists are able to "build" duplicates of many of Nature's essential complexes in the laboratory. This has happened with many vitamins. First the chemical composition is learned and the pure substance is isolated. Then a "duplicate" is made which is identical chemically and biologically with Nature's product. A vitamin is a vitamin regardless of its source just as salt is salt whether it comes from a mine or is evaporated from the sea. So efficient is large scale manufacturing that vitamins are sold at a lower cost than if they were extracted from natural sources.



The Hoffmann-La Roche people, who produce a good percent of the vitamins used in enrichment, use amazingly complex processes with scientific production controls. This requires modern, special equipment filling whole buildings, each one a city block square and many stories high.

The combination of scientific research, thorough know-how and mammoth manufacturing processes—plus the far-sightedness of leaders in the macaroni industry—is helping vitally to make good macaroni products better.

This article, reprints of which are available without charge, is published as a service to the macaroni industry by the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

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NUTRITIVE VALUE OF CENTRAL AMERICAN BEANS.

I. VARIATION IN THE NITROGEN, TRYPTOPHANE, AND NIACIN CONTENT OF TEN GUATEMALAN BLACK BEANS (*PHASEOLUS VULGARIS*, L.), AND THE RETENTION OF THE NIACIN AFTER COOKING[a,b]

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(Manuscript received September 3, 1953)

Beans are the second most common food throughout rural Central America. Despite the predominantly corn (*Zea mays*) diet of the inhabitants, clinical surveys in this region have not revealed a significant incidence of pellagra, and it was considered possible that a study of the niacin content of beans would help explain this observation. Since tryptophane can be converted to niacin in the body (*5, 11*) and a deficiency of niacin in the diet can be corrected by an increased intake of tryptophane (*26*), the tryptophane content of the beans was also determined.

Nitrogen assays were included not only to aid in the interpretation of the tryptophane values but also because of the importance of beans as a protein source. Jones *et al.* (*15*), studying beans consumed by the Maya Indians, reported that the protein of beans tends to complement that of corn due to the relatively high proportion of lysine, tryptophane, histidine, and cystine compared with the amounts found in corn.

Considering their great importance for many areas of the world, studies of the nutritive value of varieties of *Phascolus vulgaris* are surprisingly few. The problem is complicated by the enormous range of variation within the species and the fact that no satisfactory taxonomic criteria for the classification of varieties have been worked out. The nitrogen content of beans of this species grown in Latin America has been variously reported as 3.18% by Cravioto *et al.* (Mexico) (*4*), 3.94% by Munsell *et al.* (Central America) (*17-22*), 4.00% by Jaffé (Venezuela) (*13*). The average niacin content has been reported as 1.78 mg. per 100 g. by Cravioto *et al.* (*4*), 2.10 mg. per 100 g. by Jaffé *et al.* (*14*), and 2.70 mg. per 100 g. by Asenjo (Puerto Rico) (*2*). Amino acid composition data for the area are even more limited. Jaffé (*13*) reported that the essential amino acid limiting the growth of young rats was methionine in 3 *Phascolus vulgaris* and

[a] Part of these data were included by Elena Marcucci in a thesis entitled "Efecto del Cocimiento Sobre la Niacina en Frijoles Negros (*Phascolus vulgaris*, L.)" and presented to the School of Pharmacy, University of San Carlos, Guatemala. The bean selections were furnished by Dr. Irving E. Melhus, Director, Iowa State College Tropical Research Center, Antigua, Guatemala.

[b] Contribution I-15 from the Instituto de Nutrición de Centro América y Panamá.

10 other legume seeds. The digestibility of black beans was lower than that of the other legume seeds studied.

In one of the few studies on the influence of differences in environment on the nutritive value of beans, Gough and Lantz *(8)* reported that both environmental and variety factors have a very marked influence on niacin, riboflavin, and thiamine content of beans. Since the consumption of *Phaseolus vulgaris* varies from 30 to 120 g. daily per adult in the rural areas of Central America *(12)*, any marked variation in the quality or quantity of their protein or in their niacin activity should have practical importance in assessing the nutrition problems and potentialities of the area.

MATERIALS AND METHODS

Ten samples of black beans *(Phaseolus vulgaris)* from the 1950 fall crop were obtained in different regions of Guatemala and analyzed for nitrogen, tryptophane, and niacin in September 1951. They are representative of varieties considered excellent for human consumption because of their size, hardness, shiny black episperm, and good keeping quality. The raw samples were ground to approximately 20 mesh.

Moisture was determined by the AOAC standard method *(3)*. Nitrogen was determined by the Kjeldahl procedure as described by Hamilton and Simpson *(10)*. Niacin was measured microbiologically *(24)*. Tryptophane was estimated by the microbiological method of Wooley and Sebrell *(29)* with the modification that prior to the enzymatic hydrolysis the material was autoclaved at 15 pounds' pressure with 75 ml. of water for 30 minutes. The assay media were prepared according to Greene and Black *(9)*. *Lactobacillus arabinosus* 17-5 (No. 8014, American Type Culture Collection, Georgetown University Medical School, Washington) was used for the assay of both niacin and tryptophane.

The cooked samples were prepared by placing 100 g. of the raw whole beans in 800 ml. beakers with 500 ml. of water. Electrical heating units covered with wire-asbestos mats, gave uniform boiling at 96°C., the boiling point of water at the altitude of Guatemala City. Water was added to replace that lost by evaporation and the beans were stirred occasionally to avoid charring. A period of approximately 3 hours and 45 minutes was found to be sufficient for cooking under the conditions used. At the end of this period the beans were ready to be eaten although the degree of cooking was varied slightly among samples as determined by the softness of the seed. The samples were cooled, placed in a Waring blender with the cooking water, and homogenized.

The analysis of variance and determination of the correlation coefficients (r) were done as described by Snedecor *(27)*.

RESULTS

The samples varied from 5.1 to 11.1% in moisture content as received, with an average of 8.0. The nitrogen, niacin, and tryptophane contents, calculated on the basis of 10% moisture, are given in Table 1. The nitrogen variation from 2.92 to 3.64% represents a range in protein content of 18.3 to 22.8 g. of protein if the conversion factor (6.25), recommended by the U.S.D.A. *(16)* and F.A.O. *(6)*, is used. The average of 3.25% nitrogen is approximately equivalent to 20.3% protein. Upon analysis of variance the differences in nitrogen content among varieties proved to be highly significant.

The tryptophane content ranged from 0.13 to 0.19%, with an average value of 0.17%, but showed no correlation ($r = -0.081$, d. of f. 8) with the variation in nitrogen content. The variation in tryptophane among varieties was not significant.

The niacin content ranged from 2.14 to 2.53 mg. per 100 g. with an average of 2.42 mg. per 100 g. This variation did not correlate with the nitrogen content ($r = 0.092$, d. of f. 8). The inter-variety differences for niacin are statistically significant at the 5% level ($F = 3.24$, d. of f. 9, 10). The tendency of the amounts of tryptophane and niacin to be positively correlated although suggestive did not prove to be statistically significant ($r = 0.588$, d. of f. 8), possibly because only a small and relatively homogenous group of varieties was studied.

The effect of cooking on the niacin content of the 10 varieties is indicated in Table 2. The average percentage retention was 88.5, with a range of 84.2 to 95.1,

depending upon the variety. The average moisture content of 76.3% after the cooking procedure should guide dietetic calculations involving locally cooked black beans. A significant loss of niacin in the cooked samples occurred ($F = 11.59$, d. of f. 2). The difference in the niacin content among the different varieties after cooking proved to be even more highly significant than in the raw samples.

If 75 g. is taken to represent a common quantity of beans consumed daily by adults in Central America, 7 of the selections (Table 1) supply a little over 50% of the minimum daily requirement of 0.25 g. of tryptophane recommended by Rose *(25)*. The average value was 50% with a range of 40 to 56%. On the basis of the daily allowance of 15 mg. of niacin recommended by the National Research Council (U. S.) *(23)*, the varieties studied when cooked would supply from 9 to 12% of this amount with an average of 11% (Table 2).

TABLE 1

Nitrogen, tryptophane and niacin content of ten uncooked black bean samples

Selection	Calculated on the basis of 10% moisture[1]				
	Nitrogen %	Tryptophane		% requirement tryptophane in 75 g.[2]	Niacin mg./100 g.
		%	mg./g.N.		
1A	2.92	0.18	61.6	56	2.53
2A	3.64	0.16	44.0	48	2.38
3A	3.37	0.17	50.4	52	2.50
4A	3.25	0.19	58.5	56	2.39
5A	3.18	0.17	53.5	52	2.52
6A	3.51	0.17	48.4	52	2.48
7A	3.43	0.17	49.6	52	2.47
8A	3.06	0.17	55.6	52	2.38
9A	3.02	0.14	46.4	44	2.14
10A	3.14	0.13	41.4	40	2.38

[1] The figures are the average of two determinations.
[2] A value of 0.25 was taken as the human tryptophane requirement for adult maintenance *(25)*.

TABLE 2

Content and percentage retention of niacin in ten cooked black bean samples

Selection	Moisture %[1]	mg./100 g. at 10% moisture	Retention %	% allowance after cooking in 75 g. raw sample[2]
1A	73.1	2.13	84.2	11
2A	72.5	2.06	86.4	10
3A	76.2	2.11	84.5	11
4A	77.6	2.23	93.3	11
5A	79.9	2.37	94.1	12
6A	77.5	2.17	87.6	11
7A	76.4	2.08	84.3	10
8A	76.4	2.27	95.1	11
9A	74.9	1.84	85.8	9
10A	77.6	2.14	89.9	11

[1] Average of two determinations.
[2] The National Research Council (U.S.) *(23)* recommended daily allowance of 15 mg. for niacin.

DISCUSSION

Although the varieties of *Phaseolus vulgaris* studied were all shiny black and from a limited geographical area, the variation in nitrogen content was considerable. An even greater variation in nitrogen content

may be expected when a variety of shapes and colors from several countries are examined.

The complementation that may occur between corn and beans is of practical importance. Nutritional surveys have shown that corn is the most important constituent of rural diets in Central America with beans next in importance. The approximate amounts consumed per day by adults in many Guatemalan Indian communities are 500 g. of corn and 75 g. of beans *(12)*. Aguirre *et al. (1)* in a study of the nutritive value of Central American Corns have shown that this amount of corn provides an average of 0.23 g. of tryptophane and 8.8 mg. of niacin. Recent studies *(12)* indicate a loss of approximately 30% in niacin during the process of preparing tortillas from yellow corn. The daily intake of cooked beans provides an average of 0.13 g. of tryptophane and 1.6 mg. of niacin.

Adding the figures for tryptophane and niacin in both corn and beans, and applying the correction for tortillas, the diets will contain an average of 0.36 g. of tryptophane and 7.8 mg. of niacin. Goldsmith et al. *(7)* in studies of niacin requirement in man with diets of known tryptophane content suggested that with a corn diet which provides about 0.19 g. of tryptophane, the minimal niacin requirement of man is approximately 7 mg. per day. Hence, for some adults in Guatemala the combination of corn and beans provides 44% more tryptophane than the minimum requirement suggested by Rose *(25)* and 11% more niacin than Goldsmith's results would indicate to be necessary for the prevention of clinical signs of niacin deficiency. These figures may help to explain the absence of pellagra among people eating large quantities of corn in Central America.

The loss of niacin in the cooking procedure employed is statistically significant but not of great practical importance since the maximum loss is less than 16%. The variation in retention among the several varieties is of considerable interest since it demonstrates that considerations other than the original niacin content of beans can influence their nutritive value in human diets. These factors may include hardness of the episperm, size of the seed, formation of niacin-carbohydrate complexes, and other similar factors. No apparent relationship was found between percentage retention and general appearance of the seed. Variations in the size of the cotyledons in proportion to the other structures may be one factor accounting for difference in niacin content between varieties since Terroine and Desveaux-Chabrol *(28)* have shown that 96% of the niacin is contained in the cotyledons.

SUMMARY

Ten varieties of beans *(Phaseolus vulgaris)* were analyzed for nitrogen, tryptophane, and niacin. The seeds were collected in various parts of Guatemala and represented the shiny hard black type prized for human consumption. The nitrogen content averaged 3.25 and varied from 2.92 to 3.64%. The differences among varieties were highly significant. The tryptophane content averaged 0.17 and varied from 0.13 to 0.19%, but the differences among varieties were not statistically significant. The niacin content averaged 2.42 mg. per 100 g. and varied from 2.14 to 2.53 mg. per 100 g., with significant differences among varieties. All values are adjusted to 10% moisture. Although niacin and tryptophane showed a

tendency toward a positive correlation ($r = 0.588$, d. of f. 8) no statistically significant relation was found among the contents of any of the nutrients measured.

It is estimated that the corn *(Zea mays)* plus beans consumed by many Guatemalan Indian families provides as much as 0.36 g. of tryptophane and nearly 8 mg. of niacin per person per day, a fact which may help explain the absence of pellagra in Central America even though the population consumes large quantities of corn.

After 3 hours and 45 minutes of boiling at 96°C., the niacin retention averaged 88.5%. The variation in niacin among the varieties increased greatly after cooking, indicating that some varieties were more resistant to niacin loss than others.

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VITAMIN AND AMINO ACID REQUIREMENTS OF *LACTOBACILLUS PLANTARUM* FROM CUCUMBER FERMENTATIONS[a]

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The vitamin and amino acid requirements of lactic acid bacteria have been extensively investigated and have been the subject of a number of reviews; see Tittsler *et al. (8)*.

Examination of the literature reveals that there are often large differences in the requirements of various strains of the same species for these nutrients. This is quite evident in the case of *Lactobacillus plantarum* when the investigations of all the strains of the synonyms of this species (e.g., *Lactobacillus arabinosus* and *Lactobacillus pentosus*) are considered. Therefore, it was indicated that isolates of *L. plantarum* from different sources might vary widely in their nutritive requirements.

With the exception of the work by Rosen and Fabian *(4)* who demonstrated that 10 isolates of *L. plantarum* from cucumber fermentations required biotin, niacin, and pantothenic acid, no study of the vitamin and amino acid nutrition of isolates from this source was found in the literature. Therefore, four isolates obtained from four different commercial cucumber fermentations and identified as *L. plantarum (1)* were screened for their vitamin and amino acid requirements. *L. plantarum* 17-5[b] was run concurrently for comparison.

MATERIALS AND METHODS

Procedures for the maintenance of stock cultures, preparation of inocula, and for the inoculation and incubation of the test media were essentially the same as outlined by the Association of Vitamin Chemists, Inc. *(7)*, except an incubation temperature of 30°C. was used for the four isolates of *L. plantarum* from cucumber fermentations.

The medium used to test for vitamin requirements was made up from individual ingredients according to the formula given for the pantothenate assay medium of Difco Laboratories, Inc. except that 0.002 g. per liter of calcium pantothenate was added. Individual vitamins were omitted from various lots of the medium to test for the effect of their omission on the five strains of *L. plantarum*.

The basal medium I proposed by Sauberlich and Baumann *(5)* for microbiological assays using *L. plantarum* as the assay organism was used to determine the amino acid requirements of these isolates. The 5 isolates were screened for amino acid requirements in the same manner as for vitamins.

RESULTS

Vitamin requirements. As in the work of Rosen and Fabian *(4)* all strains of *L. plantarum* tested were noted to require biotin, niacin, and pantothenic acid (Table 1). Riboflavin was essential for significant acid production by 2 strains (L-20 and L-15) and appeared stimulatory for strain L-10. Three of the isolates from cucumber fermentations (L-20, L-15, and L-10) required *p*-aminobenzoic acid in this medium and strains 17-5 and L-5 were greatly stimulated by its presence. Pyridoxine and thiamine were not essential for any of the 5 strains tested but the former was very stimulatory for strain L-5.

[a]Journal article No. 1536.
[b]American Type Culture Collection No. 8014 labeled *Lactobacillus arabinosus* 17-5.

TABLE 1

The effect of the omission of various vitamins from a semi-synthetic medium on the acid produced by various strains of L. plantarum

Vitamin omitted	Ml NaOH (ca. 0.1N) to titrate acid produced by various strains				
	17-5	L-20	L-15	L-10	L-5
None	15.50	13.05	13.60	12.90	13.70
Biotin	1.60[1]	0.10	0.20	0.10	0.40
Niacin	0.30	0.30	0.20	0.30	0.45
Pantothenate	0.20	0.00	0.05	0.00	0.00
Riboflavin	14.20	0.50	0.40	9.50	14.00
p-aminobenzoic acid	4.95	0.95	0.50	0.50	7.20
Pyridoxine	11.60	11.60	12.10	11.30	6.40
Thiamine	13.05	12.25	13.15	12.20	12.65

[1]All results are underlined where the amount of NaOH necessary to titrate the acid produced was less than one-half that of the control.

Amino acid requirements. The effect of the omission of the various amino acids from the basal medium on the acid produced by the various strains of *L. plantarum* is given in Table 2. The 5 *L. plantarum* cultures were constant in their requirements for tryptophane, leucine, isoleucine, valine, glutamic acid, cystine, and threonine. Phenylalanine and tyrosine were required by three strains (L-20, L-15, and L-10), arginine by one

TABLE 2

The effect of the omission of various amino acids from a synthetic medium on the acid produced by various strains of L. plantarum

Amino acid omitted	Ml NaOH (ca. 0.1N) to titrate acid produced by various strains				
	17-5	L-20	L-15	L-10	L-5
None	11.95	9.65	9.75	9.90	10.05
L-tryptophane	0.00[1]	0.00	0.00	0.00	0.00
L-leucine	1.35	0.00	1.30	1.20	1.35
DL-isoleucine	3.30	1.30	2.90	2.75	2.60
DL-valine	0.80	0.70	0.65	0.70	0.40
L-glutamic acid	0.80	0.80	0.70	0.85	0.60
L-cystine	0.00	0.00	0.00	0.00	0.00
L-asparagine	10.70	8.50	8.40	8.70	7.55
L-lysine	11.40	8.55	9.50	8.75	10.60
DL-threonine	2.55	2.90	3.05	2.90	2.00
DL-alanine	7.75	3.20	3.75	3.55	4.85
DL-methionine	12.15	8.65	9.35	9.40	8.20
DL-phenylalanine	11.20	0.40	0.40	0.35	8.40
L-arginine	11.85	8.20	8.05	7.90	0.75
L-tyrosine	10.40	0.40	0.45	0.40	8.70
L-histidine	7.90	7.75	7.50	7.50	8.00
DL-serine	11.75	8.90	9.55	9.35	8.20
L-proline	11.30	9.00	9.45	9.05	7.95
Glycine	11.90	9.50	9.95	10.20	9.80

[1]All results are underlined where the amount of NaOH necessary to titrate the acid produced was less than one-half that of the control.

(L-5), and alanine was noted to be very stimulatory to the four isolates from cucumber fermentations. Alanine also appeared stimulatory for 17-5. Some stimulation of all 5 strains was noted with histidine. The other amino acids did not greatly influence the acid produced by these cultures.

DISCUSSION

The vitamin and amino acid requirements of *L. plantarum* isolates from cucumber fermentations were found to be similar to those for the well known 17-5 strain. The requirements of this species for biotin, niacin, and pantothenic acid and leucine, isoleucine, valine, glutamic acid, and tryptophane noted in this investigation are in agreement with the reports of many other workers. Cystine and threonine have also been observed to be essential for this organism in this work and in that by other investigators. Conversely, cystine was found by Dunn *et al. (2)* to be nonessential for *L. plantarum* 17-5 in a greatly enriched medium and Lyman *et al. (3)* and Stokes and Gunness *(6)* have definitely established that *L. plantarum* 17-5 does not require threonine in certain media. Thus, it appears that the requirements of this species for cystine and threonine depends on the basal medium used for testing.

Some variations were noted among the isolates tested in respect to riboflavin and *p*-aminobenzoic acid and to phenylalanine, tyrosine, and arginine requirements. Previous reports have also been conflicting as to the requirements of this species for these nutrients. However, since the basal medium was constant in these experiments the variations noted must be due to differences in strains rather than to the test medium.

SUMMARY

Four isolates of *L. plantarum* from cucumber fermentations and the well known 17-5 strain of this species were found to have very similar vitamin and amino acid requirements. Thus, the vitamins—biotin, niacin, and pantothenic acid; and the amino acids—leucine, isoleucine, valine, glutamic acid, cystine, tryptophane, and threonine were found to be essential in the basal media used for all strains tested. Riboflavin was essential for two strains and *p*-aminobenzoic acid for three strains from cucumber fermentations. Alanine, phenylalanine, arginine, and tyrosine were either stimulatory or essential for one or more of the isolates.

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PALATABILITY AND ASCORBIC ACID CONTENT OF BROCCOLI FROZEN UNDER DIFFERENT CONDITIONS*

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Much of the earlier literature suggests that rapid or quick freezing leads to better texture in vegetables than does slow freezing. However, later work has shown that while very rapid and very slow freezing caused texture differences in asparagus *(1)* other vegetables tested were not affected as to palatability and vitamin content *(2, 3)*.

Broccoli is one of the vegetables whose texture is most apt to be affected by freezing, the resulting product being rather limp and flabby. Accordingly, a project was designed to test the effects of different freezing rates on palatability and ascorbic acid content of broccoli, using equipment and methods which could be applied, if desirable, in home freezing of vegetables.

METHODS

Broccoli was washed and trimmed, splitting the stalks where necessary so that no piece was thicker than one inch. It was then blanched in steam for 5 minutes, and cooled thoroughly in running water.

One lot of broccoli was spread one layer deep on racks, frozen, then packaged in cellophane bags in cardboard boxes. Another lot was packed in cellophane bags, frozen, then boxed. The third lot was packaged in bag and box before freezing. One-pound packages were used throughout. One complete packaging series was frozen in still air in a home freezer set at −17.8°C.(0°F.). A similar series was frozen under the same conditions, but with a 6-inch fan placed in the freezer to keep the air in motion. Thermocouples were inserted into the broccoli stalks in representative packages to obtain temperature records during freezing.

The broccoli was tested fresh, within one week of freezing, and after 3, 9, and 13 months of storage at −17.8°C.(0°F.). After the appropriate storage, each package was cooked in one cup of boiling water plus one-half teaspoon of salt. The broccoli was scored while hot by a panel of 5 experienced judges. The samples were scored for appearance, color, odor, flavor, texture, and general acceptability, on a scale ranging from 7 *(excellent)* to 1 *(very poor)*.

Reduced ascorbic acid analyses were done by the method of Loeffler and Ponting *(4)* on the broccoli before cooking. All tests were made on duplicate packages. The data obtained were analyzed by analysis of variance.

RESULTS

The time-temperature curves are shown in Figure 1. A fan in the freezer to keep the air in motion nearly doubled the rate of freezing of the broccoli. Use of packaging materials reduced the speed of freezing, with a further reduction of speed as the layers of wrapping material increased from one to two.

The average scores for flavor, texture, and general acceptability are shown in Figure 2. Appearance, color, and odor were not affected significantly by any of the variables studied. Flavor, texture, and general acceptability varied from one storage period to another, but were not altered significantly by rate of freezing. None of the samples received low enough scores to be considered unacceptable.

*Journal article No. 1552 from the Michigan Agricultural Experiment Station.

The effects of freezing rate on palatability were so variable that the F-values for rate of freezing were nonsignificant for all scoring factors, even though there were several cases in which the scores for flavor, texture, and general acceptability increased as the speed of freezing decreased.

Percent retention of ascorbic acid in the various samples is listed in Table 1. The use of the fan to increase freezing speed did not alter the ascorbic acid retention. However, the retention tended to decrease as the rate of freezing was decreased by addition of packaging material. The broccoli frozen unpackaged had the highest ascorbic acid retention, that frozen in cellophane bag was next, and that frozen in bag and box was lowest. These differences were significant. Length of frozen storage did not affect the ascorbic acid retention significantly.

SUMMARY

The effects of varying speeds of freezing on palatability and ascorbic acid retention of broccoli were studied. Samples were frozen unpackaged,

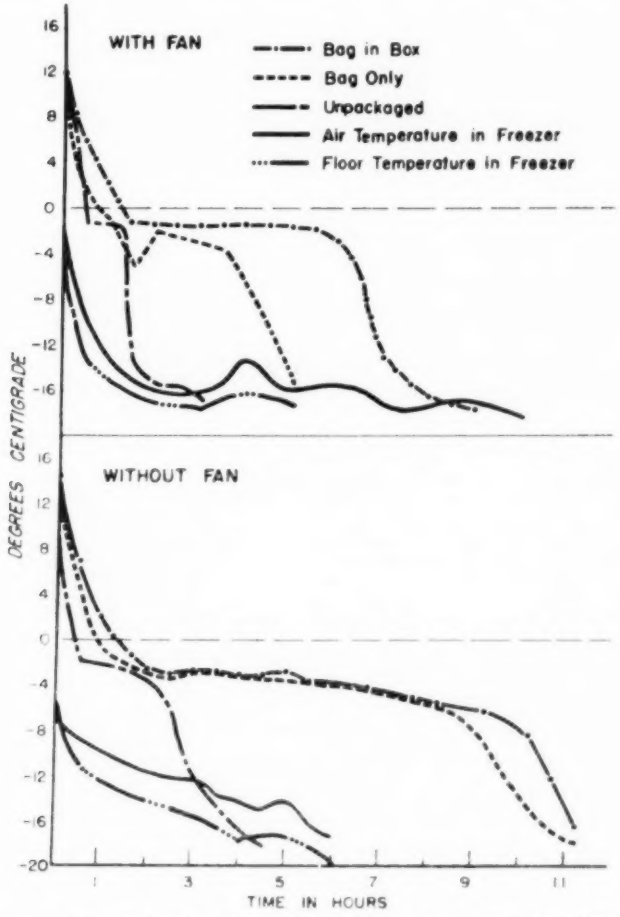

Figure 1. Rate of freezing of broccoli in freezer set at —17.8°C.(0°F.).

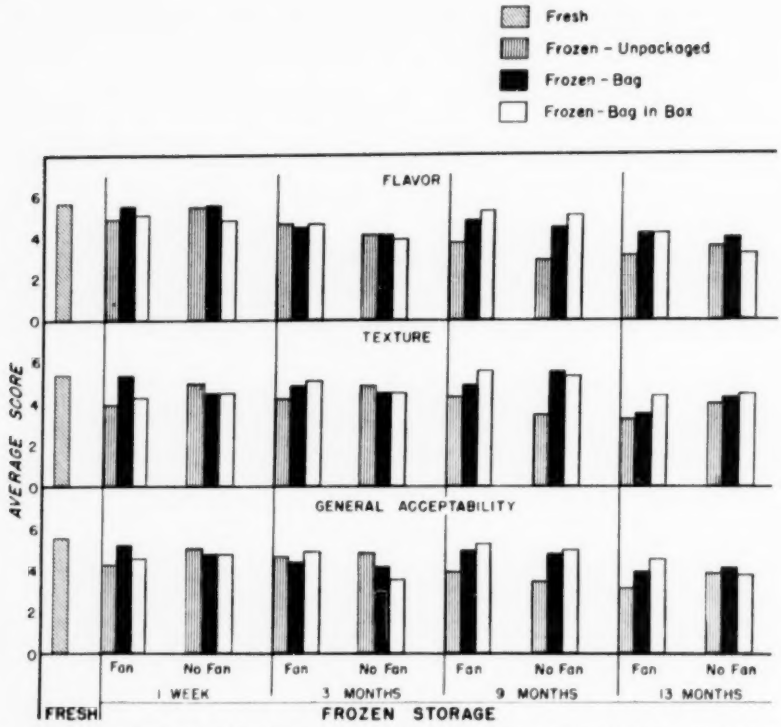

Figure 2. Average palatability scores for broccoli frozen under different conditions (maximum score = 7).

TABLE 1

Percent retention of reduced ascorbic acid in broccoli frozen at different rates

Freezing conditions	Frozen storage		
	One week	3 months	13 months
	percent	*percent*	*percent*
With fan			
Unpackaged	74.4	80.0	84.9
Bag	78.2	78.9	76.2
Bag in box	73.4	76.0	78.2
Without fan			
Unpackaged	79.2	82.9	81.5
Bag	73.8	77.0	80.2
Bag in box	75.8	76.5	72.1

Analysis of variance

Source	Degrees of freedom	Mean square	F-value
Total	17	.....	.....
Storage	2	13.03	1.76
Fan	1	0.08	<1
Package	2	40.34	5.45 *
Remainder	12	7.40	.....

* $p < 0.05$.

in cellophane bags, and in cellophane bags plus cardboard boxes, in still air and in moving air. Tests were made on the fresh broccoli, within one week of freezing, and after 3, 9, and 13 months of storage.

The conditions used caused appreciable alteration in the rate of freezing. However, palatability was not affected significantly by freezing rate. There was no indication that increased speed of freezing improved the texture of the broccoli. Ascorbic acid retention was not affected by the use of the fan to keep the air in motion during freezing, or by the length of storage. However, the ascorbic acid retention decreased slightly as the speed of freezing decreased due to the addition of packaging material.

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CRISPNESS IN ALMONDS

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"Crispness," as applied to the texture of a food product, is not a well-defined term. Crispness in one commodity may not mean the same textural quality as crispness in another, *e.g.*, crispness in fresh lettuce as compared with crispness in potato chips. Additionally, crispness even in a single commodity may be variously interpreted by different individuals, *e.g.*, crispness in nuts.

In attempting to compare varieties of almonds with regard to crispness, therefore, it becomes necessary to define this term for the purpose of the investigation. One possible definition of crispness is the quality of fracturing under relatively slight distortion, hence "brittleness." Based on this concept, an objective test (described below) was devised to measure the amount of distortion incident to the fracture of a test specimen. However, another plausible definition of crispness is the quality of fracturing into many small pieces under compressive pressure. Although such a quality of fragmentability is susceptible of organoleptic evaluation, objective measurements are pursuable only with considerable technical difficulty. Pitman *(5)* used shear strength as a measure of crispness in almonds. Shear strength perhaps may be related to fragmentability via the failure of test specimens, which are under compressive stress, in various shear planes. In addition, "hardness" may play a rôle in the concept of crispness.

MATERIALS AND METHODS

During the course of the study, 7 varieties of almonds were used: Drake, Non Pareil, Ne Plus Ultra, Jordan, Italian Seedling, Tarragona, and Marcona. All were grown in the Sacramento Valley, California. In addition, Marcona almonds imported from the Mediterranean area were examined in the organoleptic phase of the study. When raw almonds were employed, these were shelled and permitted to stand at room temperature in the laboratory until their moisture content became equilibrated with the laboratory atmosphere, a period of several months. The dry roasting method was used to produce processed almonds. In this procedure the shelled seeds were immersed for 2 minutes in boiling distilled water and then cooled in distilled water at 80°F., after which their seed coats were removed. These blanched almonds were then dehydrated for 17 hours at 145°F., with an air speed of 500-600 C.F.M. Roasting was carried out in aluminum pans in a 300°F. rotary oven for 30 minutes to give a light roast. Upon being removed from the oven, the almonds were stored in a desiccator over anhydrous $CaCl_2$ and used as needed.

Mechanical testing. At the time mechanical testing was carried out, the foreign Marcona almonds were not available. Both raw and processed seeds of the other varieties were tested. The cotyledons of each seed were separated, and from a cotyledon a bar was cut and shaved carefully to 2.0 mm. x 2.0 mm. x 15-20 mm. so that the sides of the prismatic bar were quite smooth and parallel. When roasted almonds were to be tested, the bars were cut to size before roasting. The bar was placed to protrude from a fixed horizontal groove, 2.1 mm. high, whose lower edge was curved with a 2-mm. radius. Above the groove and 5 mm. out from the center of the 2-mm. radius was fixed a micrometer, graduated in 0.01 mm. The micrometer was operated at a constant rate of speed to deflect the tip of the protruding bar until fracture occurred. The amount of deflection of the bar, from first contact to fracture, could be used directly as an inverse

index of brittleness or could be used to calculate the angle through which distortion occurred before fracture. Thirty specimens were taken for each determination.

Organoleptic appraisal. Only the roasted seeds were used for organoleptic testing. In order to minimize varietal differences in size, shape, and color, the almonds were cut transversely in halves or thirds and served under amber lighting. The factors tested were arbitrarily designated as: *fragmentability* (ability to break into many, small pieces), *hardness* (difficulty of penetration of teeth), and *cohesiveness* (requiring deep penetration of teeth before breaking apart). In addition, the judges were asked to indicate relative "crispness" between samples but were not given any formal definition to influence their selection.

A panel of 5 judges was selected from a group of 10 on the basis of a preliminary test which involved the presentation, on 2 consecutive days, of two of the varieties believed to be most different in texture. The 5 judges who were most consistent in scoring all factors and who were in agreement with the average panel scores on both days were chosen. The same method of scoring was used for the panel selection test as for the study.

A paired comparison method was employed in an incomplete, balanced block design. At each session, 2 samples were compared. Hence, for the 8 samples, 28 sessions were required to compare all of the 8 varieties with each other. Each combination of samples thus received 5 judgments, and since each sample was presented in 7 different combinations, there were 35 judgments per sample. The pairs were compared on a binary scheme by scoring the sample with the greater of each quality with 1 and that with the lesser with 0. Total scores, therefore, give an index of the ranking quality of each variety with respect to the factors scored. Correlation coefficients were calculated among the factors studied and L.S.D. values for the ranges of varietal scores for each factor computed *(7)*.

Moisture determination. Moisture-equilibrated raw and roasted almonds were ground in a Wiley-type mill to pass a 10-mesh screen. Although, generally, the particles fell through the screen, the roasted ones tended to cohere to form a granulated oily mass of "almond butter." Both butter and particles were placed in a tared moisture dish, weighed, and then dried in a 70°C. oven at 29.5 inches vacuum for 96 hours. The dry weight was determined, and percentage moisture computed.

Histological examination. Raw almonds of the different varieties were embedded in paraffin via conventional techniques, sectioned at 10 μ on a rotary microtome, and stained for microscopic observation. Sections were especially made to pass through the area of fracture so that the mechanism of breakage might be compared among the different specimens.

RESULTS

Mechanical tests. The average deflections for the bars of raw and roasted specimens in terms of vertical linear displacement and angular displacement are given in Table 1. It may be seen that there is some change in textural characteristics following the roasting process, particularly in the Jordan variety. On the whole, the roasting process seemed to bring about a change in texture toward more brittleness, *i.e.*, less distortion before fracture.

TABLE 1

Displacement in almond test bars incident to fracture

Variety	Linear displacement (mm.)		Angular displacement	
	Raw	Roasted	Raw	Roasted
Drake	0.807	0.818	9°08′	9°18′
Non-Pareil	0.915	.....	10°22′	.....
Ne Plus Ultra	0.885	0.884	10°02′	10°02′
Italian Seedling	0.916	0.872	10°22′	9°54′
Tarragona	0.915	0.909	10°22′	10°12′
Marcona (domestic)	0.949	0.896	10°45′	10°10′
Jordan	0.951	0.818	10°45′	9°18′

By using the Wilcoxon (*8*) Chi square method for determining significant differences between groups, it was found that the varietal averages were significant to less than 0.001 probability in the raw state and to less than 0.05 probability in the roasted state.

Organoleptic appraisal. The summary data for each variety are presented in Table 2, which also includes the ranges and least significant differences for each factor. This table shows that among varieties there is a significant difference at the 5% level in the fragmentability, a significant difference below the 1% level in hardness, no significant difference in cohesiveness, and a significant difference below 1% in crispness.

Since the organoleptic data appeared to show relationships among the various qualities, correlation coefficients were calculated among these qualities. These are given in Table 3. The values indicate a peculiarity of crispness evaluation. Four of the judges consistently graded the harder seed as the crisper one, whereas the fifth used fragmentability as a constant index of crispness. In the preliminary appraisal for judge selection, 9 out of 10 judges related hardness and lack of fragmentability with crispness.

Moisture content. Since added moisture generally will cause a commodity to lose its crispness, the almonds were compared as to their moisture content in the raw and in the roasted state. The moisture percentages varied between 3.42 and 4.37 in the raw almonds and between 0.50 and 0.90 in the roasted ones. These values are fairly uniform and are not particularly related to the crispness differences which have been disclosed mechanically and organoleptically.

TABLE 2

Ranking scores in organoleptic tests[1]

Variety	Fragmentability	Hardness	Cohesiveness	Crispness
Drake	11	27	22	26.5
Non Pareil	16	26	20	27
Ne Plus Ultra	18	15	15	19
Italian Seedling	17	17	18	11
Tarragona	13	20	21	16
Marcona (domestic)	14	22	22	23
Marcona (foreign)	24	9	13	10
Jordan	27	4	9	7.5
Range	16	23	13	19.5
5% level				
Range	15.40	12.10	16.50	14.85
L.S.D.	9.52	7.48	10.20	9.18
1% level				
Range	18.48	14.52	19.80	17.82
L.S.D.	13.16	10.34	14.10	12.69

[1]The values for the range indicate the difference between the highest and lowest scores for each factor tested. If this figure exceeds the value, "5% range," then the range of varietal values is significant, statistically, *i.e.*, could occur by chance less than 5% of the time. The least significant difference (L.S.D.) figures indicate the difference which must exist between any two varietal scores for those scores to be considered as significantly different, either at the 5% or 1% levels.

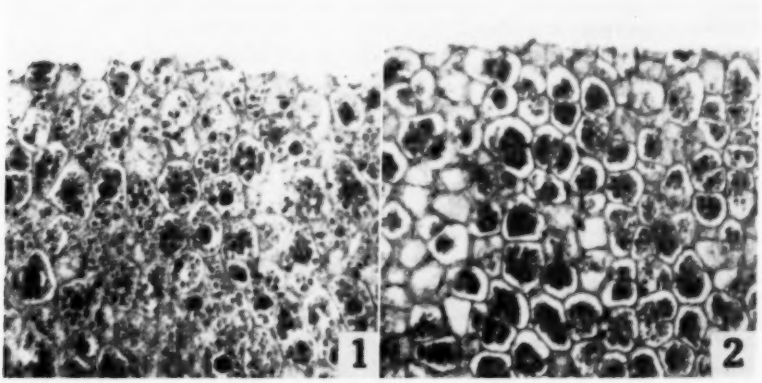
TABLE 3

Coefficients of correlation (r) among factors judged

Factors correlated	r
Fragmentability × hardness	−0.930
Fragmentability × cohesiveness	−0.966
Fragmentability × crispness	−0.778
Hardness × cohesiveness	0.945
Hardness × crispness	0.904
Cohesiveness × crispness	0.773

Histology. The bulk of the cotyledon is composed of parenchymatous tissue. The cells of this tissue are filled with large aleurone grains, each containing numerous globoids in the proteinaceous matrix. Surrounding the aleurone grains is the cytoplasm, with the oil reserve of the cell. No starch is present. The cells are relatively small, 35μ-60μ in diameter, and generally isodiametric in shape. Their walls are about 0.5μ-1.0μ thick. Small air spaces occur at the cell interstices. Throughout the parenchymatous tissue are located procambial strands, relatively widely spaced, in which little vascular differentiation has taken place. At the cotyledon surface, there are 3-4 layers of progressively smaller parenchyma cells, covered by the small, irregular cells of an epidermis. This latter lacks a cuticle.

At the fractured surfaces, the line of fracture passes across cell walls rather than between them (Figures 1 and 2). In other words, individual cells are broken when the



Figures 1 and 2: 1. Section of cotyledon of raw Jordan almond at edge of fracture surface. × 142. 2. Section of cotyledon of raw Drake almond at edge of fracture surface. × 142.

bars are fractured. Whole cells are not separated in this process. The line of fracture tends to be straight and even across the bar, perpendicular to its long axis. However, there is no cell conformation which predisposes this type of fracture. Natural cleavage lines among groups of sister cells run parallel to the long axis of the cotyledon. When larger segments are broken, there is a conchoidal type of fracture face, as is characteristic of glass, quartz, and other brittle materials.

Structural differences between varieties are not apparent by visual observation. Measurements across 100 sections of two extreme varieties (Drake and Jordan) give an average sectional cell diameter of 36.4μ for the Drake and 37.5μ for the Jordan, with no statistically significant difference between these diameters. Likewise, measurements of cell wall thickness do not show any decisive varietal differences.

DISCUSSION

The general question concerning adequate judgment of what is meant by crispness in almonds is apparently answered in the results of this study. As far as sensory perception is concerned, a crisp almond is one which does not yield in a ductile fashion under stress but ruptures sharply with very slight distortion. In the organoleptic testing, the major divergence of opinion does not appear on the above question but rather on the kind of failure which occurs in the material. Hence one view is that crispness involves a clean sharp fracture (relating crispness with hardness) and the alternate view that it involves multiple, anastomosing fractures (relating

crispness with fragmentability). It becomes apparent that an objective measurement of the kind of failure which occurs might be construed on the basis of the amount of new surface produced during failure of the specimen. The quantitative test could be based on some facet of permeability *(4)* or surface adsorption *(3)* by the commodity.

There is a general parallelism of the ranking of the different almond varieties between the mechanical tests of raw almonds and the organoleptic judgments of roasted almonds. Pitman's *(5)* values of shear strength in some of the same almond varieties likewise parallel the hardness determinations found here. Whether or not it is possible to mitigate these varietal differences by experimental treatments cannot be fully answered on the basis of the present data. The results in the mechanical test suggest possibilities in roasting alterations.

The term *cohesiveness, i.e.*, the ability to resist fracturing when placed under stress, was introduced into the tasting procedure to describe a quality usually considered the reverse of crispness. However, the organoleptic study gave *cohesiveness* a rather high positive relation with crispness ($r = .773$). The probable explanation of this relation may lie in the general association by the judges of *hardness* with *cohesiveness*. In other words, it would seem that there is some considerable practical difficulty in estimating, with the increased pressure of one's bite, whether the object is more resistant to penetration or is requiring a deeper penetration for failure to occur. The term seems to have been more difficult to analyze sensorily than was anticipated.

No clue is given histologically for the textural differences found in the almonds. It is of interest to note that fracture lines go through the cell wall. This indicates that the intercellular cement is stronger than the cell wall itself, since otherwise whole cells would be separated as they are in cooked potato *(6)* and in ripe apple *(1, 2)*. It seems reasonable to suggest that the crisp quality of the nut is related to the relative lack of moisture in the cell walls, causing them to be stiff and brittle rather than pliable and flexible as they are in the presence of moisture. The loss of crispness in dry foods which become moist is well known.

SUMMARY

A comparative study of crispness in seven varieties of almonds was made. For two quantitative definitions of crispness (amount of distortion before fracture and degree of fragmentability) objective and organoleptic measurements, respectively, were taken. Fragmentability in roasted seeds appears to be inversely correlated with amount of distortion in raw seeds and with hardness in roasted seeds. Most judges related hardness and lack of fragmentability with crispness. Varietal differences were found to be statistically significant in both the organoleptic and mechanical tests.

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STUDY ON SOME PHYSICAL PROPERTIES OF HONEY

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During recent years there has been a definite trend for beekeepers to market honey crops through cooperatives or to sell direct to honey processing plants. This practice permits proper blending to ensure uniform quality, reduces costs, and facilitates a wider distribution of honey—especially in the urban areas.

Only a few honey processing plants exist, and engineering firms have thus had little experience in their design. Since the volume of the honey industry is very small compared with other food industries such as the sugar or dairy industries, comparatively little research has been carried out for solving or improving specific engineering problems.

Some experiences of the plastic and sugar industries in handling heat-sensitive viscous liquids can be utilized. There are, however, many phases in the processing where the peculiar properties and the specific quality requirements of the honey have presented many problems not yet satisfactorily answered. Until now engineering firms have designed tubes, tanks, heat exchangers, etc. for honey processing plants more on a "hit-or-miss" basis than as a result of basic research.

In honey processing plants, large quantities of honey have to be heated or cooled and transferred from one tank to another. Furthermore, concentration and filtration are steps which have to be adapted to the special requirements of the final product. Therefore, it should be useful to have physical data based on experimental work.

HEAT CAPACITY

The amount of heat in calories required to raise the temperature of one gram of a substance by one °C. is the specific heat. It is usually determined either at constant pressure or at constant volume.

Heat capacity is dependent on the absolute temperature. This has to be taken into account if measurements are made at significantly different temperatures. Specific heat is directly proportional to temperature.

The specific heat has been determined either by the molten ice method:

$$S = \frac{80.1\ M}{mt}$$

where

M = molten ice in grams
m = material in grams
t = temperature of material in °C

or by the method of mixtures which has been found more convenient by this investigator:

$$S = \frac{(W_1 + W_2)(t_f - t_i)}{M(t_m - t_f)}$$

where

M = sample in grams
t_m = temperature of sample in °C.
W_1 = water value of calorimeter, mass × sp. heat
W_2 = mass of water
t_f = final temperature
t_i = initial temperature

Measurements were carried out in a large thermos bottle with a Beckman thermometer. The samples were kept in a stainless steel screw-cap container of about 30-g. capacity. The stirrer was motor driven and had a rubber propeller. The heat input of the latter was insignificant.

For practical reasons it has been found necessary to determine the specific heat of honey solutions at various concentrations. These series of measurements were carried out only at 20°C. (Figure 1).

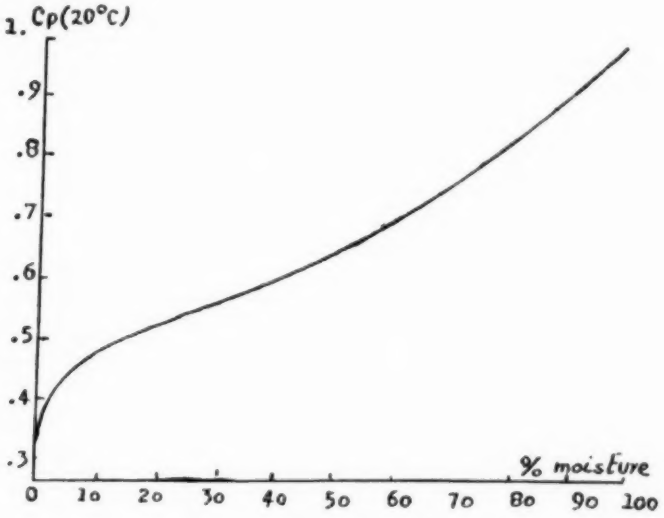

Figure 1. Specific heat of honey.

For a determination close to 0% moisture, a sample of honey was dehydrated *in vacuo* at 60°C., then kept in a P_2O_5-desiccator until constant weight was reached. The region below 15% moisture was not thoroughly investigated because of its lesser importance. The abnormality of the curve in the aforementioned region probably indicates the gradual formation of hydrates or super saturation of the solutions. This time-dependent factor must not be omitted should a more detailed investigation of this part of the curve become necessary.

The specific heat of various types of honey showed insignificant differences. The deviations were marginal to the experimental error and might be caused by the variation in the levulose-dextrose ratio in the honey.

The temperature coefficient of the specific heat of honey has been found in average and in first approximation to be

$$0.005 \text{ joules or } \sim 0.02 \text{ calories per } ^\circ\text{C.}$$

Consequently, in the temperature range as used in the honey processing industry, this factor may have an influence only on the second decimal of the specific heat.

HEAT OF DILUTION

If honey is diluted, heat will be generated. The amount of heat evolved depends on the absolute temperature of the system and the initial and final concentrations. If, to a honey solution, water of the same temperature is added a certain number of calories must be substracted to keep the temperature constant. This will be true, however, only if the initial concentration is lower than the point of saturation. At higher con-

centrations, if crystals are present in the honey or if the solution is supersaturated, heat absorption takes place by dilution. This is the heat of solution, and has a negative value for honey. Aside from the scientific interest these data are important for the determinations of the amount of crystals formed in honey.

It has been known that crystallized honey will give a different taste sensation from that of liquid honey. Furthermore, the grade of crystallization (that is the amount of crystals per unit weight) and the size of the crystals are of great importance in forming the optimal texture and flavor of honey. Small crystals are more palatable than coarse crystals, and the proper ratio between liquid and solid will determine the quality as a spread. These requirements are satisfied to the highest degree by the Dyce process *(3)*.

It became important, however, to measure the size of the crystals more accurately than just describing them as fine or coarse. Also the amount of crystals was inadequately determined by the denominations *crystallized* or *partially crystallized*.

The separation of the solid phase of crystallized honey from the liquid part is extremely difficult. At lower temperatures the honey is so viscous that neither vacuum or pressure filtration nor centrifuging is sufficiently practical. Solvents or higher temperatures cannot be applied because of the rapid dissolving of the crystals. Therefore, various indirect methods have been tried for the determination of the quantity and size of the crystals in honey.

The specific gravity of dextrose crystals differs from that of liquid honey; consequently, the measurement of the specific gravity of the partially crystallized honey had hypothetical possibilities for determining the quantity of crystals in honey. The results of these measurements, however, as obtained with pycnometer or other gravimetric methods, were inadequate.

The fact that dextrose forms a hydrate if crystallized was also utilized. The experiment was based on the thought that part of the moisture in the honey will disappear by the crystal formation of the dextrose. The original or total water content of the honey must be known, then, by filtering a small amount of the liquid phase, its moisture content can then be redetermined. This in turn will indicate the per cent of crystals in the honey, provided there is no loss of moisture by evaporation. Unfortunately, this relatively simple method was upset by the fact that the crystals formed are not only composed of dextrose but also of a variable amount of levulose. Besides, the X-ray diffraction pictures revealed strong lines not belonging to either dextrose or levulose and indicating the formation of crystals composed perhaps of dextrose and levulose as a double salt.

To solve the problem by the method of diffraction was theoretically expected on the following basis: If the honey was placed in a glass capillary in a Debye "powder" camera and X-ray photographs were taken of liquid and completely crystallized honeys, the density of the halo, caused by the non-crystalline part and measured by a microphotometer, might give an adequate figure for the ratio of liquid-solid phases. Furthermore the diffraction pattern of the crystalline part will give information on the ratio of dextrose-levulose, which is unknown. Moreover the size of crystals can be approximately computed by measuring the median peak breadth of a dextrose or levulose line.

For the determination of the liquid-solid ratio a sample was forced into a fine glass capillary and placed in a rotating "powder" camera. The temperature in the camera was maintained at 36° F., which was the storage temperature of the honey. The low temperature was brought about by cooling the camera with dry ice. After the picture was taken the crystals in the capillary were melted in a steam bath and a second picture was taken. In the latter the amorphous ring, representing the liquid phase, had a greater intensity. This method, however, gave far too inaccurate results and is not applicable for practical purposes.

Eventually it was found that the determination of the heat of dilution gave the proper answer to some of the problems, at least. The method is simple and can be carried out with the equipment of any conventional laboratory.

The heat of dilution could be determined by adding water of known temperature in a calorimeter to a honey solution of the same temperature and recording the temperature gradient (Figure 2).

If the concentration is changed between any two points of the curve (Figure 2) the heat evolved is indicated by the difference of the abscissa. If, for instance, regular honey is diluted to 3% concentration, each gram will develop about 5.5 calories.

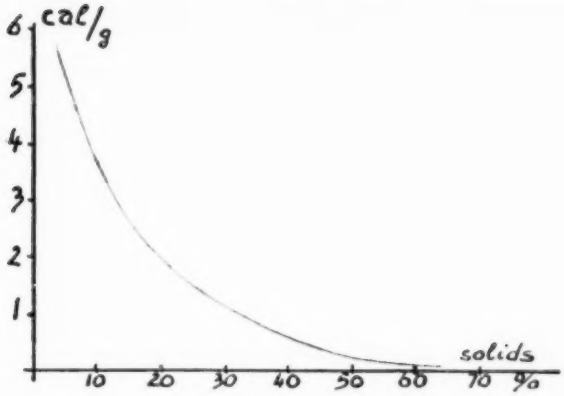

Figure 2. Heat of dilution of liquid honey.

The heat of solution is found by measuring the combined thermodynamic effect of dissolving solid dehydrated honey. By deducting the heat of dilution and extrapolation to 0% moisture content, a good approximation of the value can be achieved.

The heat of solution of one gram of crystallized honey has been found to be 673 frigors.

In consequence, the temperature gradient of partially crystallized honey by dilution will result in curves as can be seen e.g. in Figure 3.

Knowing the moisture content of honey, heat of dilution, heat of solution, and the mass of the honey, the per cent of crystals in the honey can easily be computed from such curves.

THERMAL CONDUCTIVITY

Data on the thermal conductivity of honey are of considerable interest to the industry. For design of heating and cooling units, this factor has great economic importance.

The heat transmission of solutions is dependent on their concentration and temperature. Figures on the thermal conductivity of some aqueous solutions of constant concentration at various temperatures can be found in the literature, but frequently

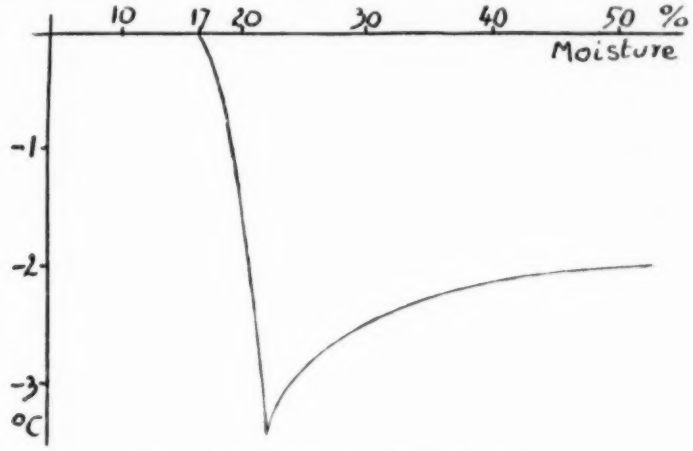

Figure 3. Heat of dilution of crystallized honey.

with great inconsistency by different authors. Even standard handbooks show contradictory figures for the thermal conductivity of such simple compounds as water. It is amazing to find, however, how few data are available in the literature so far as mixtures are concerned. These figures show a great similarity to the temperature gradient for various compounds if they have similar molecular structure. It is impossible, however, to apply thermal conductivity figures to different materials based on figures obtained by other compounds. Side chains, for instance, reduce thermal conductivity very strongly. In salt solutions the thermal conductivity is proportional to the concentration and is additive for partial ion quantities *(11)*.

In spite of its importance, the molecular mechanism of heat conduction is not yet completely understood. There is no mathematical formula developed which would enable us to predetermine the heat conductivity of any kind of solution. In addition to the Fourrier law,

$$\frac{dQ}{dt} = -KA\frac{da}{dL}$$

where $\frac{da}{dL}$ represents the temperature gradient

there are several equations used which will give an approximation of the heat conductivity of simple liquids, but they will have an error of 10-30%. For instance there is the Smith formula:

$$K = 0.043\ p\ Cp\frac{p}{M}$$

where

K = heat conductivity
p = density g./ccm.
Cp = specific heat at constant pressure
M = molecular weight

or for aqueous solution the formula *(8)*:

where

$K = K_w(1\text{-}10^{-5}\,a\,p)$
K_w = thermal conductivity of water
p = g. of solute in 100 g. solution
a = specific factor

There is an equation based on the velocity of sound and the free path of the molecules *(10)*. The quantum theoretical definition of thermal conductivity is based on the oscillation of atoms *(4)*. There are also various empirical formulas available *(11)*.

Recently, theories have been developed *(1)* according to which the transfer of heat is not an effect of the movement of individual molecules but is related to intermolecular forces or bonds. This makes the understanding of the very complex picture of the thermal conductivity of aqueous solution mixtures even more difficult.

The finding *(2)* that the thermal conductivity can be determined at a first approximation from data on viscosity, specific heat, and latent heat of vaporization might bear some practical use. For the problems in research of honey, however, this method could not be applied.

The temperature coefficient of thermal conductivity is

$$\lambda = \lambda_o(1 - a\,t)$$

where

λ = thermal conductivity cal./cm. sec. °C.
λ_o = thermal conductivity at 0°C.
a = specific factor
t = temperature °C.

which has positive or negative values for solids.

After overcoming considerable difficulties in preparing dehydrated honey crystals, it has been found that their thermal conductivity decreases about 1% per increasing

°C. This factor is generally negative for liquids except for water or aqueous solutions where it is about +0.2% per °C.

Experiments were carried out for the determination of the heat transmission of honey solutions at various concentrations. No such data could be found anywhere and the few figures on simple sugars were in the lower concentration bracket; honey contains 80-85% solids.

Radiation plays a relatively minor role in the heat transmission of honey as it is absorbed readily in a small layer of honey. Therefore, conductivity cells with inclined and highly polished surfaces could not separate radiation from conduction, and the results below indicate them together. When carrying out measurements of thermal conduction on honey solutions, an instrument has to be used with which the formation of eddies, thus convection, can be avoided.

For practical reasons it is necessary to determine the heat conductivity of liquid honey at elevated temperatures and of crystallized honey at low temperature. This has been carried out in two different cells. It was found advantageous to use a longer cell for the higher temperature range and a shorter cell for the lower temperatures.

The density of most liquids or solutions is inversely related to the temperature. In much of the equipment described in the literature, the heat energy is applied horizontally or on the bottom of the cell. With this type of heat transmission, currents and eddies are easily formed, causing convection which upsets the measurements of conduction. Therefore, in the equipment described below, the heat was applied at the top of the cell. By this means the heat passes through the sample and increases the temperature of a certain amount of water. This increment of temperature related to time, furnishes the factor of thermal conductivity of a given sample.

In cell No. 1 (Fig. 4) the heat energy was furnished by water of controlled temperature or by steam, which passes through a small copper container (H). The heat which passes through the sample is transferred by means of a copper block (Cb) in the water chamber (W), the temperature of which is always lower than that of the copper container (H). The larger the potential difference between the two containers, the smaller the error caused by imperfect equilibrium of the system. It should be kept in mind, however, that a new source of error is introduced in computing the thermal conductivity, since the arithmetical mean cannot be applied for large temperature differences. For correction a differential equation could be employed but the error can be neglected for differences of a few degrees.

The temperature increment of the water chamber is measured by a Beckman thermometer which permits readings with accuracy of 1/1000 of a °C. The method, whereby the quantity of transmitted heat is measured by the amount of ice melted in unit time, gave unsatisfactory results in this equipment.

Three copper-constantan thermocouples (T) are inserted at different levels into the cell. Their use is twofold: a. Their time-curves should be a straight horizonal line which indicates that the system reached equilibrium, and no currents appear in the cell; furthermore, it proves that the heat input is constant; b. the three figures, as rendered by the couples with the initial and final temperatures, give a fairly accurate curve of the temperature gradient in the given range and the given sample.

The results of the measurements can be seen in Figures 5 and 6.

In cell No. 2, which was designed for low temperatures and small potential differences (Fig. 7), the heat was furnished by a 100-ohm resistance coil *(4)*. The current was controlled by a variac (Var.) and a precision voltmeter (Vm.).

The sample was placed in a unit chamber (C) having a cross section of 10 cm.[2] and a height of 1 cm.[a] It is mounted between two water chambers (U. and L.).[b] The lower chamber is close to room temperature and the upper is heated a few degrees higher. The measurement is started if the temperature difference is not more than 2°C. The time is measured during which the temperature difference diminishes by 1°C. In the second method, which is used to check the results, the upper chamber is kept close to

[a] This container is drilled in a plastic disk (J) and has a copper bottom (C_T) with fins for better heat transfer.

[b] The copper bottom of the upper chamber serves as the heat transmitting lid of the sample.

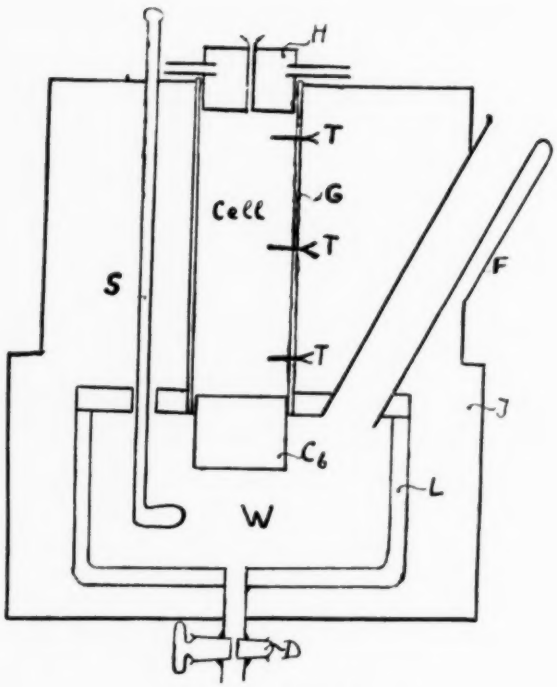

Figure 4. Cell No. 1.

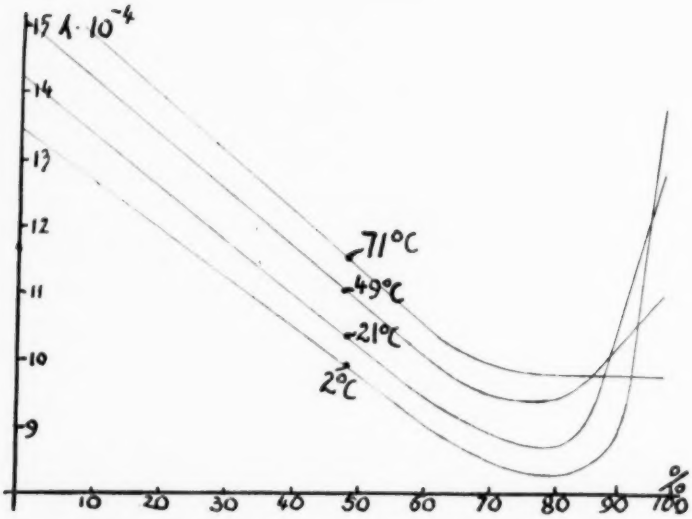

Figure 5. Isotherms of heat conductivity of honey solutions.

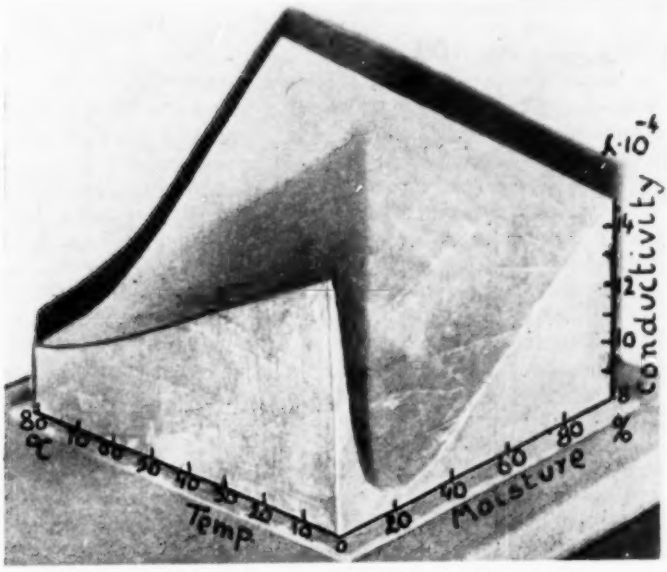

Figure 6. Illustrating heat conductivity of honey solutions.

room temperature. The current input, which is necessary to maintain this temperature if the lower chamber is filled with ice, is then determined.

The upper chamber is equipped with an effective motor stirrer (M_s and S_m) while the lower chamber has a manual stirrer (S_h). The additional mechanical heat caused by the motor-stirrer is predetermined and is taken into account. The manual stirrer is used just before each reading and its error could be neglected.

Both water chambers have calibrated copper-constantan thermocouples (T_u, T_l). Their current is fed through a two-level multiple switch (S) into the potentiometer. With this switch the temperatures of both chambers can be measured individually or the difference of the two can be read directly.

The chambers are well insulated from each other and the whole equipment is placed in a vacuum bottle (V) with an insulated lid. Under these circumstances, the temperature loss has been found to be negligible.

The equipment can be easily dismounted and reassembled (see Fig. 7). The thermal conductivity of finely crystallized honey as measured in this conductivity cell was determined at 20°C. The measurement of various samples, however, gave somewhat inconsistent results due to the fact that the crystal content fluctuates. The equilibrium in supersaturated complex systems, such as honey, is reached only after a considerable time lapse and the crystal formation is slow at low temperatures. The average figure, which will be close enough for all practical purposes, is 0.00129 cal./cm. sec. °C.

The temperature coefficient of the heat transmission of finely crystallized honey is a very complex problem; the solid phase has a negative coefficient while the liquid phase has a positive one. Furthermore, every change in temperature changes the solid-liquid ratio which reaches its equilibrium only in days. The results of a few measurements in the practical but narrow temperature range showed such slight differences that they must be considered to be within the experimental error.

All data on the preceding graphs are plotted from average figures found when using dark buckwheat and light amber basswood honeys. The observed insignificant differences might be due to the thixotropic properties of the buckwheat honey.

Thermal conductivity for beeswax at 15°C. has been found to be 92×10^{-6} cal./cm. sec. °C.

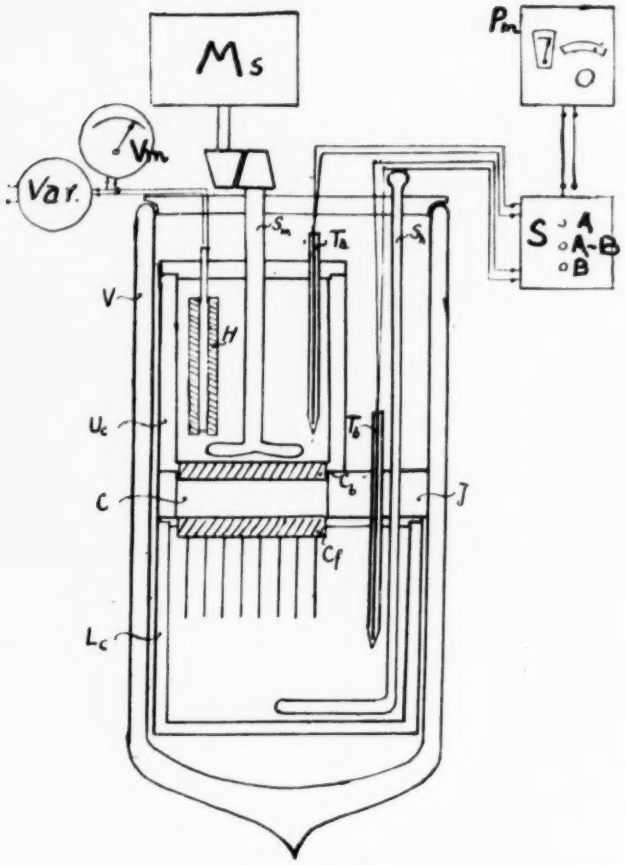

Figure 7. Cell No. 2.

FLOW CHARACTERISTICS OF HONEY

Our knowledge on the flow characteristics of non-Newtonian liquids is very limited, especially, for liquids with thixotropic properties. All equations proposed for the prediction of the flow characteristics of such liquids are empirical; but not even empirical data for honey could be found in the literature. Yet they would be very important for the processing of honey since effective cooling or heating is only possible if the flow in pipes or heat exchanger is turbulent. The exchange of the heat is much less economic if the flow is laminar.

Also the problems of viscosity and thixotropy are important factors in honey producing and honey processing. In extracting honey from the comb, letting it flow by gravity from one container into another, or in pumping it through pipes, as well as by the formation of foam, rheological laws play a major role.

Viscous flow and thixotropy are complex and very interesting phenomena. According to the dynamic theory *(6)*, in viscous flowing liquids large molecules, such as proteins, form links which have a certain elasticity and counteract the flow. If the shear strain of the flow overcomes the resistance of the links they will break, but other links will be formed again. This system is still simple enough to make the existence of thixotropy unexpected. It can be explained, however incompletely, by the theory that while standing the molecules of a thixotropic liquid will form new links created by Brownian movement. Strangely enough, thixotropy, which is destroyed by shaking or vibration of the liquid, will be promoted by laminar flow. This so-called rheopexy is explained by the orientation of the rod- or plate-shaped large molecules by streaming. Due to this organization of the particles, the formation of links is facilitated.

Thixotropy is a significant factor in heather honey and, to a lesser degree, in buckwheat honey. White or amber honeys also contain considerable amount of colloids, yet show unexpectedly low thixotropy. This might be caused by the spherical shape of the colloids or more probably by the organic acids present in the honey. The acids may adhere to the edges or ends of the colloid particles, thus interfering with the formation of a gel structure. This factor might be important in designing honey processing plants which operate with ion exchange resins.

Turbulent flow of the honey is necessary in honey processing to increase the heat exchange by convection. Such optimal flow of honey in circular pipes can be computed to good approximation with the data mentioned previously and the formula *(9)*:

$$H = \frac{24.1\,KR^{0.35}}{D^{0.36}}$$

where

H = thermal conductivity coefficient between honey and inner wall of the pipe in B.t.u./hrs. × sq. ft. × °F.

K = thermal conductivity of stagnant honey in B.t.u. × ft./sq. ft. × hrs. × °F.

$$R = \text{turbulence factor} = \frac{UDS}{M_t}$$

where

U = average velocity of flow in ft.³/sec./ft.² of cross section

D = inside diameter of pipe in inches

S = specific gravity of liquid

M_t = viscosity of honey at t temperature in poises

This empirical formula will give accurate figures if D is 1 to 4 inches and R has values 0.4 to 16. Turbulent flow, however, occurs only if R is larger than 40. In this region the above formula renders approximate values. If R is less than 12 the flow is viscous and between 12 and 40 unsteady.

To achieve turbulent flow the stream of honey must have a relatively high Renolds number, which in turn requires over-sized pumps and much energy. On the basis of theoretical speculation this investigator thinks that, similarly to the adiabatic effect of sound waves in gases, the laminar layers of relatively slowly flowing viscous or unsteady liquids will be

broken up into turbulent flow if the liquid column is exposed to an oscillating vibration of proper amplitude and frequency. This will counteract thixotropy too. The longer the heat exchanger the larger must be the amplitude of the oscillation. The frequency will depend on the viscosity of the liquid and the rate of flow. The necessary effect can be applied through pulsing or rotating valves or vibrating membranes. This needs, of course, experimental verification.

SUMMARY

It has been found that the specific heat of honey solution depends on the concentration and results in an S-curve between the specific heat of water and that of honey solids. Honey with 17% moisture has a specific heat of 0.54 if measured at 20°C. There are slight differences among various types of honeys.

The heat of solution of dehydrated honey was found to be 673 frigors at 20°C. per g.; when one g. of honey containing 85% solids is diluted with water to 3% solids, 6.5 calories are developed, provided both honey and water have a temperature of 20°C.

The amount of crystals in finely crystallized honey was determined by measuring the temperature gradient upon dilution with water.

The thermal conductivity of finely crystallized honey has been determined as about 129×10^{-6} cal./cm. sec. 0°C., and that of beeswax is 96×10^{-6} cal./cm. sec. °C. The heat transmission of a honey solution and its temperature coefficient is plotted on a three-dimensional graph.

Finally, the flow characteristics of honey are discussed.

Acknowledgment

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COMPOSITION OF BUTTERNUT SQUASHES FROM VINES TREATED WITH MALEIC HYDRAZIDE*

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During recent years numerous investigations have been undertaken to determine the possibility of regulating the rate of development of economic plants. Various chemicals have been sprayed onto fruit trees and small fruit bushes to delay blossoming until the danger of late frost has passed. The foliage of root crops, such as potatoes, onions, carrots, and beets, have been sprayed with a variety of substances to inhibit or prevent the root crops from sprouting during winter storage. Attempts have been made to eliminate undesirable plant growth such as suckers on commercial tobacco plants. Studies have shown that the rate of physiological processes in harvested crops may be retarded by chemical treatments: for example, sugar beets stockpiled awaiting processing may also lose as much as a pound of sugar per ton per day, but spraying the sugar beet foliage two to six weeks before harvest with maleic hydrazide slowed down the metabolic processes and conserved the sugar content of the beets. The literature contains references to the application of maleic hydrazide for all the conditions noted above, but few data regarding the composition of fruits and vegetables produced by plants previously treated with maleic hydrazide have been found. This study was undertaken to determine the composition of Butternut squashes from vines that had been sprayed with maleic hydrazide a short time before harvest.

EXPERIMENTAL

The Butternut squashes (*Cucurbita moschata Duchesne*) in this study were from vines raised on Connecticut valley alluvial soil, which was treated with a commercial 10-10-10 fertilizer at the rate of 900 pounds per acre. The vines were grown under normal commercial cultural conditions including frequent dusting, with an insecticide containing neutral copper (7% metallic copper), rotenone, and prophyllite, during the early growing season to repel the various bugs that infest squash vines.

The climatic conditions during the growing season were: rainfall, 17.99 inches; 761 hours of bright sunshine; average temperature, 69° F.; average relative humidity, 76.5%. The rainfall during the 41 days previous to harvesting the squashes was 6.8 inches, which was distributed as follows: August 16, 1.75 inches; August 21, 0.45 inches; September 1, 1.80 inches; September 3, 0.10 inches; September 15, 0.65 inches; September 19, 1.88 inches; September 23, 0.10 inches; and September 26, 0.07 inches.

On September 6 the squash field was divided into subsections, and 2 concentrations of maleic hydrazide, 0.05% and 0.25%, were sprayed at the rate of 160 gallons per acre on the experimental plots; other plots were reserved as controls. Apparently the maleic hydrazide solutions did not damage the squash vines seriously, for the foliage remained nearly normal. On September 16 and 26, the squashes were removed from the vines. Thus, each concentration of maleic hydrazide solution was sprayed onto squash vines 10 days and 20 days before the squashes were harvested.

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As every squash grower well knows, from midseason until killing frost every squash vine in the field carries, simultaneously, fruit of all stages of maturity from the unopened flower bud to the mature squash. Accordingly, in a study of the growth, composition, yield of marketable crop or other factors related to edible squashes, selection of the material for study is inevitable. In this instance Dr. O. H. Pearson, a plant physiologist of long and wide practical experience, selected squashes of the same degree of maturity and size. The squashes were taken to the laboratory, and 6 groups as described in Table 1 were arranged for winter storage.

Sixty squashes from each group were stored for the winter in darkness on shelves not touching each other. During the 95 days of storage the temperature of the space in which the squashes were housed averaged 64°F., and the average relative humidity was 53%. At the beginning of the storage period typical squashes were selected from each of the 6 groups for analysis for water, protein, total sugars, starch, and carotene. After the squashes had been in storage for 95 days, typical squashes were again selected from each group for assay. The assays were made by the methods adopted by the Association of Official Agricultural Chemists *(5)*.

RESULTS AND DISCUSSION

The detailed results of the assays of the Butternut squashes at harvest-time and after 95 days of winter storage are reported in Table 2. In a previous study, Holmes, Spelman, and Wetherbee *(2)* found that the

TABLE 1

Data relative to spraying Butternut squash vines with maleic hydrazide solutions

Group	Spray date	Spray concentration	Harvested
		%	
A	9/6/52	0.05	9/16/52
B	9/6/52	0.05	9/26/52
C	9/6/52	0.00	9/16/52
D	9/6/52	0.25	9/16/52
E	9/6/52	0.25	9/26/52
F	9/6/52	0.00	9/26/52

TABLE 2

Composition of Butternut squashes from vines treated with maleic hydrazide

Sample	Water %	Protein[1] %	Total sugars[1] %	Starch[1] %	Carotene[1] mg./100 g.
	Beginning of storage period				
A	86.2	10.0	37.0	23.9	19.6
B	82.6	9.1	37.4	31.6	19.0
C	85.8	7.8	45.1	28.9	19.0
D	82.1	9.5	42.5	26.8	16.2
E	78.3	6.9	40.6	27.7	16.5
F	84.1	6.3	41.5	25.8	21.4
	After 95 days of winter storage				
A	86.3	12.4	66.4	4.4	32.1
B	83.2	8.3	61.3	7.7	26.8
C	86.6	9.0	59.0	3.7	19.4
D	84.2	10.1	57.6	7.6	19.6
E	87.1	9.3	62.8	7.0	17.8
F	84.5	11.0	67.1	4.5	29.7

[1]Dry basis.

carotene and total sugar content of individual mature Butternut squashes at harvest time varied significantly. Accordingly, when the 6 groups of squashes in this study were assembled, care was exercised to select squashes similar in size, maturity, and general external appearance. However, in spite of this precaution, it is quite evident from the results reported in Table 2 that the composition of the squashes in the various groups varied significantly.

The average composition of the 6 groups of squashes at harvest time was water 83.2% and, on a dry basis, protein 8.3%, total sugars 40.7%, starch 27.4%, and carotene 18.6%; the average composition after 95 days of storage was water 85.3% and, on a dry basis, protein 10.0%, total sugars 62.3%, starch 5.8%, and carotene 24.2%. The increase in water content from 83.2% to 85.3% during storage indicates that the gain of water by respiration slightly exceeded the loss of water from the squashes by evaporation. Since the total sugars increased from 40.7% to 62.3% during the 95 days of storage and the starch decreased from 27.4% to 5.8% during the same period, it is evident that the starch was converted to sugar quite rapidly under the storage conditions maintained in this study. In his studies of physiological changes in Butternut squashes, Phillips *(7)* found, on a dry weight basis, 14.97% of total sugars at harvest time, 25.99% after two weeks of storage, and 43.55% when the squashes had been in storage 3 months. Regarding the high sugar content, Phillips says, "The relatively large amount of sucrose in Butternut after storage is striking. This may be one factor in the very desirable flavor of this variety."

The 30% increase, 18.6 mg. per 100 g. of squash to 24.2 mg., in carotene content during 95 days of winter storage indicates the rate of physiological activity of the squashes after they have been harvested. Similar results have been obtained with carrots. Lachman *(3)* stored 7 varieties of carrots on November 1 at 32°F. On April 1 the carrots were assayed a second time, and all varieties were found to have increased appreciably in carotene content after they had been stored. McKillican *(4)* and Brown *(1)* also reported an increase in the carotene content of carrots during storage. Miller and Covington *(6)* found that the carotene content of sweet potatoes increased during storage: for example, at harvest, the carotene content was 51.0 mg. per g., after one month of storage 75.3 mg., at 2 months 81.9 mg., and at 3 months 80.2 mg. of carotene per g. The average protein content of the 6 groups of squashes at harvest, 8.3%, increased to 10.0% after 95 days of storage—an increase of 20%. This increase is comparable to the 19% increase (6.54% to 7.80% protein) reported by Phillips *(7)*.

It should be noted that assays of the squashes and their seed for maleic hydrazide gave the following results: for the fleshy portion of the squash, Group A, *nil*; Group B, *nil*; Group C, *nil*; Group D, 3.8 p.p.m.; Group E, 12.3 p.p.m.; and Group F, *nil*. The seed produced by Group E contained 29.9 p.p.m. maleic hydrazide. It is hoped that additional data in this regard will be obtained from future studies.

Judged by the results obtained in this study, spraying the squash vines with maleic hydrazide 10 or 20 days before harvest did not change the composition of the Butternut squashes significantly as regards water, protein, and total sugars. The amount of starch remaining, after 95 days'

storage, in squashes of Groups B, D, and E is definitely larger than that of groups A, C, and F. The squashes of Group B were from vines treated with a 0.05% solution of maleic hydrazide 20 days before harvest and the squashes of Groups D and E were from vines sprayed with a 0.25% solution. If this difference in starch content is significant, then it indicates that the rate of physiological activity of the squashes of Groups B, D, and E was decreased as a result of preharvest spraying the squash vines with maleic hydrazide. Such a result is very greatly desired for it would serve to lengthen the storage life of Butternut squashes and it would help to conserve somewhat the food value of the squashes which is lost when starch is converted to sugar and subsequently to water and carbon dioxide. In this connection it may be noted that Wittwer, Sharma, Weller, and Sell *(18)* obtained almost complete inhibition of sprout growth in carrots held in storage 3 months by preharvest spray with maleic hydrazide and Paterson, Wittwer, Weller, and Sell *(12)*, Marshall and Smith *(7)*, Highlands, Licciardello, and Cunningham *(3)*, and Kennedy and Smith *(5)* inhibited storage sprouting of Cobbler, Pontiac, Green Mountain, Kennebec, Katahdin, and Sebago varieties of potatoes by preharvest spraying of the potato vines. Also Sweetman *(15)*, Paterson, Wittwer, Weller, and Sell *(12)*, Marshall and Smith *(7)*, and Solunkhe, Wittwer, Wheeler, and Dexter *(14)* have commented upon the feasibility of improving the color and quality of potato chips by inhibiting the conversion of starch to sugar in stored potatoes by a preharvest foliage spray of potato vines. Hansen *(2)* reported that sugar beets stockpiled for processing may lose up to a pound of sugar per ton per day—a yearly loss of about $1,500,000. Wittwer and Paterson *(17)* sprayed sugar beet foliage with maleic hydrazide 2 to 6 weeks before harvest and reduced sugar loss to less than 1% as compared with a 12% loss in the untreated controls during 35 days of storage. Mikkelsen, Griffith, and Rifle *(10)* increased the sucrose content 6.2% and Wittwer and Hansen *(16)* obtained a 10% increase in the sucrose content of sugar beets by applying maleic hydrazide as a preharvest foliage spray.

It is, of course, obvious that the results obtained here are insufficient to justify any final conclusion regarding the extent to which a preharvest spray of Butternut squash vines with maleic hydrazide will prolong the storage life of the squashes and retard the conversion of starch to sugar and the sugar to carbon dioxide and water. More data regarding the value of maleic hydrazide for prolonging the storage life of Butternut squashes are urgently needed since increasing the shelf life of Butternut squashes would permit greater use of this valuable material for human food.

SUMMARY

Butternut squash vines were sprayed with 0.05% and 0.25% solutions of maleic hydrazide 10 and 20 days before harvest. Typical squashes from the two control and the four experimental groups, taken at harvest time and after 95 days of storage, were assayed for water, protein, total sugars, starch, and carotene. The results of the assays did not show any consistent effect of the maleic hydrazide treatment on the water, protein, and total sugar content of the squashes. The rate of conversion of starch to sugar and ultimately to carbon dioxide and water was slower for squashes from

the treated vines. Considered on a dry weight basis, the protein, total sugars, and carotene content of the squashes increased during the winter storage—the starch content decreased very materially.

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SAMPLING PORK LOIN FOR COOKING TESTS*

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In meat research the most obvious sources of variation in the experimental material are the different animals used and separate muscles within each carcass. Differences among animals are usually so distinct that the need for a number of animals to represent the population is generally accepted. The various muscles in the animal body are also obviously different from each other in many ways. It is with the purpose of minimizing variations inherent in different portions of meat from one animal that many investigators use but one cut or one muscle for study.

Sampling procedures for studies of freezing, thawing, cooking methods or other treatments, in which this one cut or muscle is divided and portions assigned to different treatments must take into consideration the uniformity of the cut throughout its length.

The loin of pork, a cut of substantial size, is composed essentially of one muscle, the *longissimus dorsi*, on which tests may conveniently be performed. Small portions of the loin cut would be suitable for many studies. Chief among the advantages would be a saving of expense since 18 or more large chops can be cut from one loin.

This paper reports a trial of the uniformity of chops cut from different portions of the pork loin.

If it should be demonstrated that the cuts of the loin are essentially the same throughout its length, displaying no gradient for a given characteristic, small samples could be selected without regard for position within the loin, and each treated as representative of the whole. Thus, sampling and the assigning of portions of meat to various treatments for study would be simplified.

There is some experimental evidence that all characteristics of the loin of pork may not be the same throughout its length. The study of Satorious and Child *(2)*, in which pork rib roasts were compared with adjoining loin roasts, indicated that press fluid, total moisture, and ether extract did not differ, but total nitrogen and total cooking losses were slightly higher for the loin ends. They suggested that rib and loin sections be rotated in an experiment. Child and Moyer *(1)* found that pork roasts from different sections of the loin had different amounts of press fluid, but no gradient was observed either for the separate roasts or for successive slices within a roast. Weir *(4)* demonstrated that tenderness varied according to location of the sample in the pork *longissimus dorsi* muscle.

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PROCEDURE

Three pairs of pork loins were used, each being cut into one-inch chops which were numbered consecutively beginning with No. 1 at the rib end. Chops from one loin of each pair were cooked while fresh. Those from the matching loin were frozen, to be cooked at a later time. These chops were wrapped individually in waxed locker paper, then frozen and stored at 0°F.(−18°C.). Chops from Hog X were stored for 3½ months, those from Hog Y, 3 months, and those from Hog Z, 5 months. Thus, storage period varied between different groups, but was a constant within a given group of chops.

Each chop was roasted individually in a covered pan at an oven temperature of 253.4-255°F.(123-124°C.) until the interior temperature of the chop was raised from an initial temperature of 68°F.(20°C.) to 185°F.(85°C.).

The following observations were made on each chop: weight loss during storage, cooking time, cooking losses, shearing strength and press fluid. Shearing strength was determined on the cooked meat by means of a shearing apparatus similar to the Warner Bratzler machine. The percent of press fluid was calculated from the loss in weight of a 2.00-g. sample of cooked lean meat, pressed at 8,000 p.s.i.g. for 5 minutes at a temperature of 104°F.(40°C.).

The analysis of variance *(3)* provides a method for segregating the sources of variation—in this case, animals as well as location within each loin from which chops were taken. The analysis of variance was computed from the combined data on fresh chops from 3 hogs. The correlation coefficient measures the consistency with which the variates keep in step *(3)*. Correlation coefficients were calculated for chops from each loin separately to determine trends due to position of chops in the loin. In addition all data were plotted to detect trends other than straight line relationships.

RESULTS AND DISCUSSION

Average values for fresh and frozen chops from the rib and loin ends are shown in Table 1. By means of the analysis of variance which was calculated for fresh chops only, it was found that differences associated with different animals were highly significant for all cooking losses, press fluid, and shearing strength, Table 2. Differences associated with position

TABLE 1

Means of pork chop characteristics

Hog identification	Treatment	No. of chop	Loss of wt. during frozen storage	Cooking time	Cooking losses		Shearing strength	Press fluid
					Evaporation	Drip		
				min.	*%*	*%*	*lbs.*	*%*
X	Fresh	1 (rib)	....	84	1.0	8.1	27.5	56.0
		18 (loin)	....	86	1.6	11.9	29.5	49.4
Y	Fresh	1 (rib)	....	69	1.7	15.6	14.4	59.4
		18 (loin)	....	67	1.7	13.7	18.4	58.6
Z	Fresh	1 (rib)	....	83	1.1	11.1	22.2	55.8
		18 (loin)	....	74	1.5	15.8	24.6	53.0
X	Frozen	1 (rib)	1.8	86	1.3	9.6	21.2	55.0
		18 (loin)	2.2	75	1.2	12.5	25.7	54.0
Y	Frozen	1 (rib)	2.6	68	1.8	12.6	14.3	58.4
		18 (loin)	2.2	63	1.4	13.0	14.7	59.0
Z	Frozen	1 (rib)	0.4	73	1.7	12.5	20.4	53.4
		18 (loin)	0.5	73	1.1	15.9	23.3	54.5

TABLE 2

Analysis of variance of differences among pork chops due to hog and to position of chops in the loin

Pork chop groups	Variation	D.F.	Mean squares[1]				
			Cooking losses			Shearing strength	Press fluid
			Evaporation	Drip	Total		
Fresh chops	Hogs	2	1.229**	120.68**	133.75**	810.11**	80.51**
	Position	17	.085*	4.25	4.10	14.62**	5.41**
	Error	34	.040	2.24	2.52	4.93	1.76
	Total	53	.....	.....	.....	.....	.....

[1] ** Significant at 1% level; * significant at 5% level.

of the chops in the loin were press fluid, shearing strength, and evaporation loss during cooking. Since storage period was not identical for the chops from different hogs, the variation due to hogs could not be ascertained for frozen chops.

Correlation coefficients, calculated for each group of pork chops, to determine whether a linear relationship existed for any characteristic from the rib to the loin end, are shown in Table 3. Low numerical values of r indicate that there were no significant linear trends with successive chop number for the following: loss of weight during frozen storage, cooking time to raise the interior temperature from 68°F.(20°C.) to 185°F.(85°C.), evaporation loss during cooking, and shearing strength. The differences due to chop position found by means of the analysis of variance for cooking loss and shearing strength were therefore due to fluctuations among chops rather than to linear trends.

Press fluid showed little relationship to chop number except in the case of Hog X, fresh chops, for which a negative correlation was found. Drip loss and total cooking losses were highly correlated with chop number both for fresh and frozen chops from Hogs X and Z. Chops from Hog Y did not show this trend.

These analyses indicate that each cut of meat is likely to display differences, not only because of variation among animals and variation among

TABLE 3

Correlation coefficients for pork chop characteristics as related to position of chop in the loin
Values of "r"[1]

Hog Identification	No. of chops	Treatment	Loss of weight during frozen storage	Cooking time	Cooking losses			Shearing strength	Press fluid
					Evaporation	Drip	Total		
X	21	Fresh	....	.22	−.07	.70**	.69**	.29	−.85**
	20	Frozen	.03	.01	−.002	.87**	.85**	.14	−.37
Y	21	Fresh	....	.33	−.41*	.13	.07	.41	.18
	18	Frozen	.04	−.38	−.42	.57*	.51*	.20	−.25
Z	21	Fresh	....	−.43	.37	.87**	.89**	.40	−.42
	21	Frozen	.07	−.38	−.49*	.78**	.78**	.07	.02

[1] ** Significant at 1% level; * significant at 5% level.

separate muscles of the carcass but because of differences in the various portions of the cut itself. For some characteristics a trend may occur throughout the length of the cut.

If no trend occurred, the variations within a cut could be considered random, and each portion could be regarded as representative of the whole. In this way each consecutive portion could be assigned to a different treatment. However, trends have been shown to occur, as for cooking losses which increased from rib to loin end for Hogs X and Z. In such cases one portion is not comparable to another. The sampling procedure for pork loin chops must therefore take into consideration positional differences among chops throughout the loin. These positional effects can usually be handled by means of an appropriate experimental design.

SUMMARY AND CONCLUSIONS

The uniformity of chops cut from different locations in the pork loin was tested. Cooking time, cooking losses, shearing strength, and press fluid were determined on each chop from three fresh and three matching frozen pork loins. In addition, weight loss during storage was determined on chops which had been frozen.

By means of the analysis of variance and correlation coefficients it was demonstrated that pork chop characteristics differed, not only due to variations among animals, but due to position of the chop within the loin. In some cases, linear trends from rib to loin end were noted.

Allowance should be made for positional effects by means of an appropriate experimental design.

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ENZYMATIC DARKENING OF SHRIMP[a]

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The continued expansion of the shrimping industry within recent years and the location of new shrimp beds farther off shore than the old sources of supply have intensified the need for understanding and control of "black spot" in shrimp. This is a condition in which fresh shrimp develop a black coloration which starts in the head and then spreads to the tail where it forms black bands outlining the sections of the tail region. Unless they are iced immediately, the blackening may begin within a few hours after the death of the shrimp. Even with continuous icing, the blackening of whole shrimp may be far advanced before the catch can be marketed. Shrimp boats must remain at sea from several days to as much as two weeks to make the trip to the more distant shrimp beds.

The enzymatic nature of this blackening was not recognized until very recently, although the presence of the enzyme tyrosinase in *Crustacea* was reported as early as 1930 *(15)*. The importance of this enzyme in bringing about hardening and darkening of the cuticles of certain insects and of the crayfish and lobster was pointed out by Dennell in 1947 *(4, 5)*. Fieger in 1951 *(6, 7)* presented the first evidence for the enzymatic nature of the blackening reaction in shrimp, and stated that the enzyme is distributed in the head, fins, legs and shell but not in the tissue of the tail. The blackening could be retarded by limiting the oxygen supply, as by covering with refrigerated water, or by dipping in sodium bisulfite. Further work from the same laboratory *(1)* established that bacteria were not instrumental in bringing about the blackening.

Tyrosinase is very widely distributed in the plant as well as the animal kingdom *(3, 12)*. The darkening of raw fruits and vegetables is mainly due to this enzyme *(11)* and the literature on methods of retarding enzymatic darkening in plant tissues is voluminous. If it could be established definitely that the blackening in shrimp is due to this enzyme, measures found effective in inhibiting the enzyme from other sources might be adapted to the control of black spot in shrimp. The work here presented was undertaken to obtain further evidence for the enzymatic nature of black spot and to explore methods of retarding the reaction in shrimp.

EXPERIMENTAL

Source of material. *Penaeus setiferus* (L.), commonly known as the white shrimp, was the species used in these experiments. Whenever possible they were caught off Alligator Point with the help of the personnel and facilities of the Oceanographic Institute, Florida State University. This arrangement was ideal in that the shrimp could be put into the test solutions immediately. However, the number of shrimp obtainable at any one time with the limited facilities available was generally not great enough,

[a] This paper is derived from work originally reported in a thesis submitted by Marian B. Faulkner to the Graduate Council, Florida State University, in partial fulfillment of the requirements for the degree of Master of Science.

so it was necessary to obtain shrimp for many experiments from wholesale dealers at the docks at Apalachicola. Purchases were made of the freshest shrimp obtainable on the days when fishing vessels had docked. However, the exact age of the lot of shrimp purchased could not be determined, as it was usually composed of a mixture of the catches of several days. Shrimp were transported to Tallahassee packed in crushed ice, similar to the manner in which they are stored aboard shrimp boats and for the most part were treated in the manner described in the particular experiment immediately upon arrival at the laboratory.

For some experiments shrimp heads rather than whole shrimp were used. They could be frozen when fresh and stored in the freezer until used. The head portion blackens in a relatively short time. This was an advantage in preliminary investigations of the reaction and its inhibition. It has not been established whether the rapid darkening of the head parts is due to a greater concentration of enzyme or of substrate, or both.

Cuticle removed from fresh shrimp tails was used for the preparation of extracts containing the enzyme. The extracts were prepared by covering 200 g. of the washed cuticle (as removed from the shrimp along with any adhering membrane) with one liter of water, allowing the mixture to stand for a period of 24-48 hours in the refrigerator, then filtering. The pH of the extracts was 8.3-8.4. They darkened spontaneously when aerated at room temperature for approximately 24 hours. The darkening did not occur if the extracts were heated to the boiling point.

Observations on the Enzymatic Character of the Reaction and the Nature of the Black Pigment

The addition of various substrates to shrimp cuticle extracts. The enzyme tyrosinase is known to oxidize ortho dihydroxyphenols directly. The quinones resulting from this oxidation polymerize to give pigments which vary in color with the nature of the substrate. The enzyme will also oxidize several monohydroxyphenols, including tyrosine and para cresol, but these must first be converted by the enzyme to dihydroxy compounds. This initial conversion proceeds at a much slower rate than the subsequent oxidation of the dihydroxy compound, but the conversion is accelerated by simultaneous oxidation of small amounts of the dihydroxy compound *(2)*. The oxidation of tyrosine proceeds through various intermediates to brownish black "melanin" pigments. The oxidation of para cresol produces a reddish color *(14)*.

Since dihydroxyphenols (gallic acid, catechol) oxidized spontaneously at the high pH of the shrimp cuticle extracts even without the enzyme, only monohydroxyphenols were investigated. Their effect on time required for darkening of cuticle extracts was tested in the following manner. Fifty ml. aliquots of the extract were added to equal volumes of .002 M solutions of ortho cresol, para cresol, and tyrosine. (The tyrosine was heated slightly to bring it into solution and then cooled before adding the extract.) In the control, water was substituted for the phenol solution. Air was bubbled through all solutions at a constant rate and time required for first visible darkening recorded as follows:

Solution	*Darkening visible in*
Control	20-24 hours
Ortho cresol	20-24 hours
Para cresol	20 minutes
Tyrosine	5 hours

Para cresol with boiled extract showed no color after 24 hours.

The fact that the extract from shrimp cuticle causes color formation with tyrosine and para cresol in a comparatively short time indicates the presence of tyrosinase.

Spectrophotometric behavior of black pigment from shrimp heads. In an attempt to determine whether the dark colored pigment obtained from shrimp heads was the same as that produced by the oxidation of tyrosine by cuticle extract, the absorption spectra of the 2 pigments were compared as follows:

One hundred g. of shrimp heads were covered with 200 ml. of distilled water and refrigerated for approximately 12 hours. The dark colored solution was filtered and the filtrate diluted to bring it within an appropriate range for readings on the Beck

man spectrophotometer. The product obtained by the oxidation of tyrosine by cuticle extract as described in the previous experiment was similarly treated.

Absorption curves in the visible region were similar for the pigments from these two sources. Absorption progressively increased from longer to shorter wave lengths. However, the pigments from both sources were unstable and nonhomogeneous. The solutions continued to darken as they stood and eventually became cloudy. When the insoluble pigment was filtered off, the solutions again progressively darkened. The slope of the absorption curves obtained from different solutions, or from the same solution at different times, varied greatly. The ratio of the absorption at 400 mμ to that at 700 mμ in three experiments ranged from 3.5 to 5.0 for the pigment from shrimp heads and 2.8 to 4.5 in solutions of oxidized tyrosine. The black pigments obtained were evidently not of fixed composition, but the behavior of the pigments from the two sources was the same.

The strong absorption at shorter wavelengths was utilized in later experiments as a rapid quantitative measurement of the degree of darkening of various solutions in contact with shrimp. The solutions were filtered and read immediately in the Evelyn colorimeter using a 420 mμ filter, the shortest wavelength filter available.

Bleaching with hydrogen peroxide. While the enzymatic reaction may be retarded by a number of factors, only hydrogen peroxide has been demonstrated to have a bleaching action on the animal melanins after the black pigments have formed *(19)*. The bleaching takes place only at higher concentrations; lower concentrations have been shown to accelerate the reaction *(18)*.

To test the effect of hydrogen peroxide on the shrimp pigment, partially blackened heads were covered with various concentrations and stored in the refrigerator, with results as follows:

0.03% H_2O_2	acceleration of blackening
0.3 % H_2O_2	acceleration of blackening
3.0 % H_2O_2	complete bleaching in 2-3 hours

Visible evolution of gas at the two lower concentrations indicates decomposition of the hydrogen peroxide, thus supplying additional oxygen to the system. The blackening in the presence of dilute hydrogen peroxide is uniform throughout, whereas controls darken progressively from surface to interior. The shrimp treated with 3% hydrogen peroxide were very attractive in color; even when blackening was far advanced they could be restored to the light condition of newly caught shrimp. However, the accelerating effect on blackening of lower concentrations of the reagent excludes treatment with this reagent as a preventive measure even if it were otherwise suitable.

EXPERIMENTS ON THE CONTROL OF BLACKENING

Control measures which have proved most successful for retarding enzymatic darkening in cut fruits and vegetables include the use of reducing agents such as ascorbic acid, the control of pH by the addition of citric and other acids, and treatment with sulfurous acid and its salts *(11)*. All of the above treatments were found to retard the darkening of shrimp heads or whole shrimp when covered with solutions of the inhibitors and refrigerated.

Ascorbic acid. Ascorbic acid is able to reduce quinones formed upon the oxidation of dihydroxyphenols and so prevent their polymerization to dark pigments *(16)*. In the present study, ascorbic acid and sodium ascorbate, in concentrations ranging from 0.05 to 0.5%, were very effective in preventing darkening of cuticle extracts in the presence of added phenolic substrates as well as inhibiting blackening of heads or whole shrimp as long as they remained immersed in the solutions. However, these compounds showed little retarding effect when shrimp were dipped in the solutions for periods up to an hour, then refrigerated dry. Work with these compounds was discontinued and will not be reported here in detail because of their relatively high cost coupled with the fact that they were not effective as a surface dip.

Lowered pH. The values reported in the literature for optimum activity of the enzyme tyrosinase have varied widely *(3, 12, 19)*. Apparently many factors can affect this optimum, such as source of the enzyme, substrate used, and even the buffer system

in which the tests are carried out *(16)*. In a number of studies, optima between pH 6 and 8 have been recorded, but in some cases activity may continue to increase or to remain at a high value up to pH 9.5. In general, the activity is progressively decreased as the pH is lowered below pH 6.

Preliminary tests were carried out on the effect of various acid solutions, including hydrochloric, phosphoric, acetic and citric, in preventing darkening of shrimp heads. In these experiments approximately 100 g. of shrimp heads were covered with 200 ml. of the solution being tested. The mixtures were stored in covered beakers in a refrigerator at 5°C.(41°F.). Blackening of the solutions took place first at the surface. The entire solution was poured off the shrimp heads, mixed and filtered before making pH and transmission measurements.

Since the shrimp themselves have a high buffering capacity, the pH of the acid solutions used to cover them was not constant but increased progressively with time. If the original pH of the added acid was too low, the shrimp showed some pinkening, as in early stages of cooking. This color change has been shown to be caused by protein denaturation, resulting in the freeing of the carotinoid pigment astacin, which in the fresh shrimp is bound to protein *(9)*. Since a somewhat similar pinkening is characteristic of shrimp which have been kept too long without refrigeration, such a condition would be considered undesirable. Strong mineral acids such as hydrochloric had too low initial pH and too little buffering capacity. The tribasic acids, citric and phosphoric, and their acid salts, were more effective in maintaining the pH within a reasonable low range, but even with these acids, if the proportion of shrimp to solution was large, the pH shifted very rapidly.

Table 1 shows the results of 2 experiments in which citrate and phosphate buffers were used to vary the pH. The concentration of anions was constant at all pH values except in the untreated sea water sample, i.e. the buffers were prepared by mixing various proportions of 0.1M acid and the corresponding basic salt. Since the 2 experiments were performed at different times, with different lots of shrimp, the values obtained with citrate are not strictly comparable with those obtained with phosphate.

In both experiments the most rapid rate of darkening (as shown by the lowest transmission values) was obtained in the unadjusted sea water samples, in which the pH varied between 7.5 and 7.9 during the storage periods. As the pH was decreased below 7 progressive inhibition of the blackening occurred. There was also a lesser and nonprogressive inhibition as the pH was increased above 8. To be sure that the greater darkening obtained in the sea water samples was due to the pH rather than to a specific inhibiting effect of the phosphate and citrate ions, another experiment was performed in which the pH of a citrate buffer was adjusted to that of the sea water sample (ranging from 7.3 to 7.9). The rate of blackening was practically identical in presence and absence of the citrate.

In Table 2 are recorded the results of several typical experiments in which whole shrimp were immersed in sea water or in various acid phosphates. Sea water itself gives considerable protection against blackening. Except for the surface layer, the

TABLE 1

Relation between pH and darkening of shrimp heads covered with buffer solutions

Experiment number	Buffer system used	Original pH of buffers	pH of solutions and heads after 24 hrs.	Transmission at 420 mμ after 24 hrs.
		8.4	8.5	24
	Citrate	8.2(seawater)	7.6	4
I	0.1 M	5.8	6.6	29
		3.8	4.7	40
		2.9	4.0	65
		11.9	10.9	33
	Phosphate	9.0	8.1	30
II	0.1 M	8.2(seawater)	7.8	1
		4.5	6.2	44
		1.9	4.4	80

TABLE 2

Effect of acidifying shrimp on blackening and other spoilage symptoms[1]

Exp no.	History of shrimp	Proportion of solution to shrimp	Solution used to cover shrimp	pH range	Surface blackening of solution	Appearance of other signs of deterioration
I	Commercial lot 1-4 days old	1,000 ml. solution to 700 g. shrimp	None (dry refrigeration).	.....	2 days[2]	Blackening, main type deterioration.
			Sea water	8.2-7.8	6 days	Odor of H_2S at 4 days.
			0.2M NaH_2PO_4	6.1-6.5	22 days	Shell rough 2 days, flesh soft, H_2S odor at 14 days
II	Fresh shrimp put into test-solutions just after catching	5 to 8 parts solution to 1 part shrimp	Sea water	8.2-6.9	6 days	Odor of H_2S at 6 days.
			.05M H_3PO_4	2.8-6.4	14 days	Pinkening. Shell rough, H_2S at 14 days.
			0.1M NaH_2PO_4	5.4-6.3	14 days	Shell rough. H_2S at 14 days. Flesh not examined.
III	Commercial lot 1-4 days old	750 ml. solution 1,000 g. shrimp	Sea water	7.7-7.1	7 days	H_2S at 2 days; very strong at 5 days.
			H_3PO_4(0.2M)[3]	3.5-6.2	13 days	Shell rough, flesh soft at 10 days.

[1]All solutions stored in a refrigerator at temperatures of 5°C.(41°F.).
[2]Blackening of shrimp rather than solution in this case.
[3]Added in small increments over a 4-day period to hold pH below 6.0.

shrimp themselves do not usually show noticeable blackening in less than 2 weeks. Other forms of spoilage, especially the production of hydrogen sulfide, detectable both by odor and by the darkening of lead acetate paper, limit the storage life of shrimp held in sea water. Idyll *et al. (10)* also observed off odors in shrimp held in refrigerated sea water, although hydrogen sulfide was apparently not produced in their experiments.

Acidification of the covering solutions with acid phosphates practically eliminated all blackening, even at the surface of the solution, for extended periods. Furthermore the production of hydrogen sulfide or other spoilage odors was greatly retarded. On the other hand, several other defects appeared in the acid treated shrimp. Within a few days the shells lost their normal smooth, slippery feel and became rough. Of greater importance, the flesh tended to soften more rapidly in the acidified samples, producing a crumbly texture on the external surface of the shelled shrimp. This texture change was the most prominent sign of deterioration in the acidified samples. Since the defect developed gradually it was difficult to determine exactly when it first appeared. At the times specified in Table 2, the texture change was sufficiently far advanced that the shelled, raw, acidified samples felt definitely softer than those in sea water. However, significant differences in texture of the cooked shrimp were usually not apparent at this time. In spite of this texture defect, the acidified samples were superior after the first week of storage to those in sea water, and these in turn were much superior to those refrigerated dry at the same temperature. However, none of the refrigerated samples, whether dry or covered with various solutions, were equal in flavor after 5 days of storage to shrimp of the same lot frozen immediately and held in the freezer for the same period.

The effect of sulfur dioxide. Sulfur dioxide and various bisulfites have been used extensively in the food industries to control both enzymatic and non-enzymatic browning. Alford and Fieger *(1)* used a solution of sodium bisulfite as a dip to retard blackening of shrimp. In the present study, the effect of sodium bisulfite has been tried alone and with phosphoric acid.

Again, the preliminary experiments were done with shrimp heads rather than whole shrimp. Some shrimp heads were immersed in the solutions for the time stated. Others were allowed to remain in the solution for 10 minutes, then drained and stored in the refrigerator dry for the remainder of the storage period. Transmission readings were made on the filtered solutions.

The results of 3 typical experiments are shown in Table 3. The sulfur dioxide has the advantage over acidification and all other preventives so far tried in that it gives protection to heads dipped for a short time in the solution and then refrigerated dry.

TABLE 3

Effect of SO_2 on blackening of refrigerated shrimp heads

Exp. no.	Time of storage	Solution used to cover shrimp	Observations at end of storage period		
			pH	Transmission at 420 mμ	Appearance of dipped heads refrigerated dry
I	16 hrs.	Sea water	8.05	28	Blackened
		1% SO_2	6.2	60	Very good protection
		0.1M H_3PO_4+ 0.1% SO_2	4.4	89	Good protection
II	24 hrs.	Sea water	7.75	4.5	Blackened
		0.1% SO_2	7.7	33	Slight protection
		0.1M H_3PO_4+ 0.1% SO_2	5.8	65	Good protection
III	21 hrs.	Sea water	7.8	1	Very black
		0.1M H_3PO_4	4.4	81	Slight protection
		0.1% SO_2	6.4	55	Slight protection
		0.1M H_3PO_4+ 0.1% SO_2	5.1	85	Good protection

Dipping the shrimp in acid solution, even at very low pH values, gave only slight protection when the shrimp were removed from the solution. Combinations of bisulfite with acid were more effective than either alone, both as covering solution and also as a dip.

Since the use of sulfur dioxide in food products is strictly governed by the Food and Drug Administration, it was of interest to determine the amount of sulfur dioxide remaining in the shrimp, both raw and cooked, after various dippings and storage periods.

The method used to measure sulfur dioxide is an adaptation of the direct iodine titration method widely employed on fruits and vegetables *(17)*. It is based upon (a) liberation of sulfur dioxide from complexes in the food by allowing it to stand in an alkaline medium, (b) acidification, followed immediately by (c) titration of the sulfur dioxide with a standardized iodine solution. Blank determinations on aliquots of the sulfur dioxide treated shrimp were obtained by the addition of formaldehyde, which forms a complex with sulfur dioxide.

The detailed procedure as finally adopted for shrimp was as follows: two 10 g. samples of shrimp (A and B) were blended in 100 ml. of distilled water in a Waring blender. The contents of the blender were washed into a 300 ml. Erlenmeyer flask with another 100 ml. of water. To each aliquot were added 2.5 ml. of 5N NaOH, and the mixture was allowed to stand for 20 minutes. At the end of this time, 3.5 ml. of 5N HCl were added. Sample A was titrated immediately with standardized iodine (approximately .02N) using 10 ml. of 1% starch as an indicator. To Sample B, the blank, were added 10 ml. of 36% formaldehyde. It was allowed to stand for 5 minutes, then titrated as above. The sulfur dioxide content of the shrimp was calculated as follows: SO_2 in p.p.m. = (ml. 0.02N iodine for sample − ml. iodine for blank) × 64.

The method offers some difficulties in that the end point is not very sharp and requires some practice to be reproducible. Also, it is probable that there are substances other than sulfur dioxide normally present in the shrimp which reduce iodine and which are slowly bound by formaldehyde, since if the formaldehyde is allowed to remain in contact with the shrimp, the titration value decreases over a period of some hours and becomes much lower than blanks obtained by the titration of samples untreated with sulfur dioxide or formaldehyde. Recovery of known amounts of sulfur dioxoide mixed with shrimp samples in the blender ranged from 90% to 96% in nine trials.

In the experiments summarized in Table 4, the whole shrimp were dipped in the specified sulfur dioxide solution. They were then stored in the refrigerator for the time period stated, headed, shelled and washed before analyzing. Samples to be cooked were dropped into an equal weight of boiling water (slightly more than sufficient to cover), and cooked for 5 minutes after the temperature reached 85°C. The water did not boil during the cooking time. This cooking procedure was the minimum heat treatment necessary to firm the protein, eliminate the raw taste, and develop the pink color in medium-sized shrimp. All samples, including blanks, were analyzed in duplicate or triplicate.

TABLE 4

SO_2 remaining in shrimp after sulfur dioxide treatment

	Conc. of SO_2	pH	Time of dip	Storage time after dip	SO_2 remaining	
					Washed raw	Cooked 5 min.
			(min.)	*(days)*	*(p.p.m.)*	*(p.p.m.)*
Experiment I	1%	8.2	10	0	160	10
Experiment II	1%	8.2	30	1	....	30
	1%		20	1	35	15
	1%		20	2	10	10
Experiment III	0.1%	5.0	10	1	75	10

While significant amounts of sulfur dioxide remain in the washed raw shrimp after shelling, the amount remaining in the cooked shrimp was negligible when the dipping time was not greater than 10 minutes. No difference in flavor could be detected between sulfited and unsulfited shrimp.

DISCUSSION

While the enzymatic darkening of fresh shrimp may be inhibited by (a) limiting the oxygen supply as by holding in refrigerated solutions rather than on ice or dry refrigeration, (b) lowering the pH, and (c) dipping in bisulfite solutions, especially at lowered pH values, the application of any of these methods to the practical preservation of fresh shrimp on board the shrimping vessels, during the subsequent shipping and marketing, or in the household, offers difficulties.

Tanks and refrigeration equipment for storing shrimp in refrigerated circulating set water are commercially available. When shrimp are stored in such tanks, blackening is not of primary importance as a cause of spoilage; instead off-odors, possibly of bacterial origin, become the limiting factor. Lowering the pH of the storage water affords greater protection against blackening and retards the production of hydrogen sulfide and off-odors of a putrefactive nature. However, continued immersion in acidified waters introduces new problems, especially the early softening of texture. The cause of this texture change has not been traced. It is possible that digestive enzymes from the shrimp itself are more active at the low pH.

The use of slightly acidified bisulfite dips as a preliminary to storage either in ice or refrigerator seems to offer possibilities for convenience and economy of manipulation and prolonged protection. However, the desirability of the use of sulfur dioxide needs careful evaluation from a nutritive standpoint. It is known to destroy thiamin *(13)*. Although it can prevent the nonenzymatic browning due to condensation of amino acids and sugars, it does not prevent the early stages of this reaction which result in loss of biological availability of the amino acids *(8)*. At present, sulfur dioxide and bisulfites are used exclusively for the preservation of certain fruits and vegetables, especially as a preliminary to dehydration. These foods are not important sources of protein or thiamin but rather of ascorbic acid and carotene, which are protected by the sulfur dioxide. Extension of the use of sulfur dioxide to animal protein foods such as shrimp should probably not be recommended until much more information is available on the extent of its penetration into the shrimp and the nature and extent of nutritive losses brought about.

SUMMARY

Further evidence has been presented in favor of the view that the darkening of fresh shrimp, commonly known as "black spot," is due to formation of melanin pigments by the enzyme tyrosinase. Extracts from shrimp cuticle oxidize para cresol and tyrosine. The dark pigments obtained from the oxidation of tyrosine by cuticle extracts behave spectroscopically like those obtained from spontaneous blackening of fresh shrimp. Hydrogen peroxide, which is known to decolorize melanin pigments, bleaches blackened shrimp.

The darkening may be controlled by immersing the shrimp in solutions which protect them from oxygen, by adjustment of pH, and by use of bisulfite dips. Other types of spoilage, characterized by off-odors or texture changes, predominate when the blackening is prevented by these methods. The use of bisulfite raises nutritional problems which are discussed.

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SALT TOLERANCE OF MARINE BACTERIA

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Salting is the most widely practiced method of preserving fish in India. The preservative action of salt has been explained by Shewan *(13)* and Halvorson *(7)*. It has been stated by ZoBell *(16)* that "marine" bacteria are very sensitive to hypertonic conditions and that very few grow in sea water to which 12% NaCl is added, and virtually none in 24% NaCl. Castell and Mapplebeck *(4)* noted that unlike marine micrococci which tolerate high amounts of salt, other bacteria, especially *Flavobacter*, are not very salt tolerant. Castell *(3)* also showed that *Clostridium sporogenes* did not grow in 10% NaCl. Buchanan and Fulmer *(2)* recorded that in concentrations of 6 to 10% NaCl, growth was inhibited in various species of meat-spoiling bacteria, *Sarcina* requiring 15% for inhibition. Hammer *(8)* records that putrefactive bacteria were more sensitive to salt than other types and that the inhibitory action was especially pronounced at a concentration of 20 to 25%. As against this, Jones *(9)* found that *Bacillus cereus* tolerated 10% NaCl in immersion brines, the flora of which was mainly *Micrococci*. Haines *(6)* also found more micrococci in pickling brines tolerating high salt concentrations. Karaffa-Korbut *(11)* had noted that *B. mesentericus* grew in 12% NaCl and that asporogenous rods are viable in 20 to 25% salt. Spore formers were reported to have survived a concentration of 26% NaCl for 4 months. The living range for saprophytic bacteria, according to the same author, was 10-12% NaCl. Baranik-Pikowskyi's work has shown that even in high salt concentrations some bacteria remain not only viable but are physiologically active *(1)*. That life is possible at high salt concentration is proved by Willansky *(15)* and Elizari-Volcani (5), who isolated bacteria from the Dead Sea (28-29% NaCl). Likewise Lefevre and Round found the majority of organisms in scum of cucumber brine tolerated 25% NaCl *(10)*.

Salt tolerance of the bacterial flora of fish has to be taken into account in evaluating the efficacy of salt-curing. Hence this investigation on the salt tolerance of common fish-spoiling bacteria was undertaken. The present work confirms the findings of the above authors.

MATERIAL, METHODS AND RESULTS

The bacteria used in these experiments were 134 of the pure strains isolated from mackerel and sea water. Growth in media without salt was also noted. Growth in media of various concentrations of NaCl was studied by adding the requisite quantity of NaCl to nutrient sea water broth or agar. The percentage indicated here represents the grams of NaCl added to 100 cc. of the nutrient broth or agar medium. The cultures were incubated at 37°C. Growth was noted at 24 hours, 48 hours, and after 1 week.

The results of a study of 134 strains showed that all the *Micrococci*, *Bacillus*, *Sarcina*, and *Bacterium* species grew as well in fresh water media as in sea water media. Sixteen *Achromobacter*, 4 *Flavobacter*, 2 *Pseudomonas*, one *Corynebacterium*, one *Paracolobactrum*, and one *Alcaligenes* failed to grow without salt and are therefore strictly "marine" forms by ZoBell's definition. Among the "marine" *Achromobacter*,

one tolerated 30%, three 25%, five 15%, two 10% and five 7.5% of NaCl. The two *Pseudomonas* tolerated 15% NaCl, while all the 4 "marine" *Flavobacter* did not tolerate any salinity higher than that of sea water. The *Corynebacterium* also tolerated only 3.5%, the *Paracolobactrum* 10%, and the *Alcaligenes* 30% NaCl.

The remaining 109 strains grew in both sea-water and fresh-water media. The salt tolerance of these strains in each genus is given in Table 1.

TABLE 1

Salt tolerance of bacteria from sea water and mackerel capable of growing in media without NaCl

Genus	Number of species tested	Number of strains tolerating the maximum NaCl concentration of							
		3.5%	5%	7.5%	10%	15%	20%	25%	30%
Achromobacter	10	1	....	3	1	1	1	....	3
Micrococcus	21	....	....	4	8	8	1	....	....
Bacillus	45	2	3	9	9	10	1	7	4
Sarcina	14	....	....	....	12	2	....	....	....
Corynebacterium	2	....	....	....	....	1	1	....	....
Bacterium	3	1	....	....	2	....	....	....	....
Paracolobactrum	2	....	....	2	....	....	....	....	....
Alcaligenes	1	....	....	....	1	....	....	....	....
Flavobacterium	11	4	....	3	1	....	....	2	1
Total	109	8	3	21	34	22	4	9	8

DISCUSSION

From the data, it is seen that *Achromobacter* are generally tolerant of high salt concentrations, 17 of the 26 tolerating 10% or more, while 7 grew in 25% or more of NaCl. This is contrary to ZoBell's finding that marine bacteria are not tolerant to hypertonic salt concentrations *(16)*. On the other hand *Flavobacter* are stenohaline, more than 50% of these failing to grow in salinity higher than that of sea water. This substantiates ZoBell's *(16)* findings regarding salt tolerance of marine bacteria and Castell and Mapplebeck's *(4)* findings regarding poor salt tolerance by marine *Flavobacter*. *Micrococci* and *Sarcina* have a wide range of salt tolerance. They tolerate higher salt concentrations and at the same time are also found to grow in fresh-water media devoid of salt. Thirty-one of them tolerated 10% or more while only 4 failed to grow in NaCl media above 7.5%. The higher limit seems to be 15% and only one grew in 20% NaCl. This is not surprising in view of the fact that they are very often found in pickle-brines and other salted goods *(6, 9)*.

The tolerance by *Bacillus* for high concentrations of salt is a significant feature and its implications in fish preservation are important. Thirty-one out of 45 *Bacilli* tolerated 10% or more of NaCl. In fact, the pigmented *Bacilli* even retained their color in higher salt concentrations contrary to Sperlich's findings *(14)*. Halvorson *(7)* has opined that Gram-negative bacteria seem to be much more sensitive to NaCl than Gram-positive ones. This is almost true as is seen by the high salt tolerance of Gram-positive cocci and sporeforming *Bacilli* and the poor salt tolerance of *Flavobacter*. Marine *Achromobacter* are fairly salt tolerant. Sanborn *(12)* found that *Micrococci* and *Sarcina* in pickled fish and brine were "marine" forms. Some proved to be halotolerant and the cocci were resistant to 10-20%

NaCl. The abundance of cocci and *Bacilli* in salted goods is explained by the high salt tolerance of these organisms.

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HISTOLOGICAL OBSERVATIONS ON FAT LOCI AND DISTRIBUTION IN COOKED BEEF[a,b]

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The significance of fat as a factor influencing certain rather important organoleptic characteristics of meat is well recognized. Although it is generally known that some intramuscular fat is lost from meat during cooking, information as to the mechanism of such loss is not available. These considerations plus the important fact that determinations of organoleptic characteristics are always made on cooked meat clearly point to the need for investigating the nature of changes in adipose tissue effected by the cooking process. The present study was, accordingly, an initial attempt to trace the fat in broiled beef steaks by a technique proven capable of demonstrating all the fat *in situ*, namely, by staining frozen sections with specific fat dyes, avoiding the use of fat solvents during the processing.

MATERIALS AND METHODS

Cooked samples of *Longissimus dorsi* (ribeye) and *Semitendinosus* (round) from 9 carcasses were used (2 light-weight prime, 2 light-weight good, 2 heavy-weight prime, 2 heavy-weight good, and 1 commercial cow). A total of 111 histological samples were taken; the distribution of these with respect to aging period of these two muscles was as follows:

Aging Time in Days

	0	14	28
Ribeye	47	20	8
Round	15	11	10

At least 2 slides were made of each sample and many of these slides were stained by 2 methods. The results presented, therefore, are based on the study of more than 300 slides.

Cooking procedure. Steaks, 1½ inches thick, were broiled in electric ovens equipped with Variac transformers for precise temperature control. Thermocouples were used for determination of internal temperature and oven temperature (400°F.) at the broiling surface of the steak. Steaks were placed on individual broiler pans, broiled to an internal temperature of 100°F., turned, and broiled to a final temperature of 150°F. They were then cooled at room temperature, wrapped in aluminum foil, and stored overnight at 45°F. before histological samples were taken from them.

Histological techniques. Samples approximately 1½ cm. thick were frozen onto the nose of a Spencer rotary microtome by use of the conventional CO_2 attachment. Longitudinal sections cut at 10-15 μ in a cold room (20°F.) were transferred from the knife directly to albuminized slides, allowed to thaw at room temperature, and fixed for 10 minutes in formalin vapor.

[a] This work was conducted at the American Meat Institute Foundation, and was supported in part by Research and Marketing Funds under contract with the United States Department of Agriculture. A preliminary account of this study was published (*17*).

[b] Journal Paper No. 55, American Meat Institute Foundation.

[c] Deceased.

Two equally satisfactory methods for staining fat were standardized in our laboratory. The first was a modification of the Smith-Mair technique for Nile blue sulfate *(1)*.

Stock solution:

1 g. Nile blue sulfate (National Aniline, NNb$_4$)
4 cc. glacial acetic acid
96 cc. distilled water
Filter before use.

Staining procedure:

1% Nile blue sulfate stock solution	5-15 min.
Rinse in distilled H_2O	1 min.
Differentiate in 4% acetic acid	5- 7 min.
Rinse in distilled H_2O	2 min.

Mount in Apathy, or syrup of acacia.

Results: Fats and lipoids stain bright red or pink; remaining tissue, blue or greenish blue.

The second method was a modification of the alkaline-alcohol Sudan IV technique *(6)*. This procedure was standardized as follows:

Stock solution:

A. 2 g. Sudan IV (National Aniline, NZ 16)
70 cc. absolute alcohol
B. 2 g. NaOH (C.P.)
30 cc. distilled water
Mix solutions A and B to make up staining solution which is filtered each time before use.

Staining procedure:

Place slides in 75% alcohol	2 min.
Stain in alkaline Sudan IV	10 min.
Rinse in 75% alcohol	2 min.
Rinse in distilled H_2O	2 min.

Mount as for preceding method.

Results: Fat stains red to orange against a completely unstained background.

Both above techniques were employed for each sample until it was demonstrated that comparable results were given by either. Because the contrast of preparations stained with Nile blue sulfate was particularly suitable for photomicrography, the majority of sections studied were stained by this method.

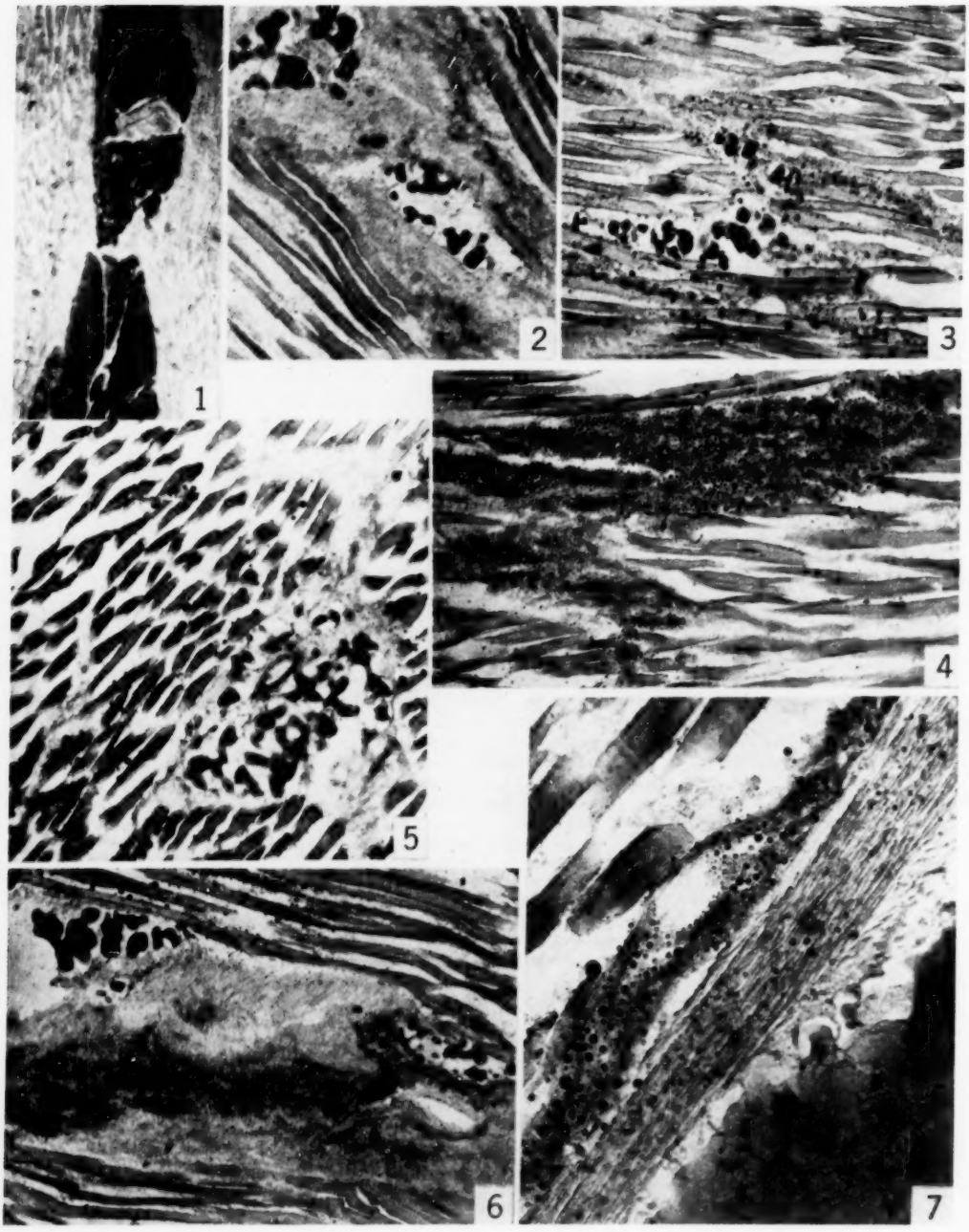
Photomicrography. Representative sections were photomicrographed in black and white on 4 x 5-inch Kodak Super-XX panchromatic films with the use of a Kodak wratten filter No. 61. The photomicrographs of Figures 1-7 are from sections stained with Nile blue sulfate.

RESULTS

The movement of fat from fat cells to perimysial spaces and the concomitant changes in the physical form of this fat will be described. This process of fat translocation was similar in cooked ribeyes and rounds from all 5 classes of carcasses. Since no such phenomenon has been found to occur in raw beef, the fat translocation can be attributed to the conditions under which the steaks were broiled. One of these conditions is obviously temperature.

Escape of fat from fat cells. Release of fat from fat cells was observed to take place in individual endomysial[4] fat cells as well as in much larger fat islands in perimysia of all sizes. No apparent relationship between the size of fat island and the amount of fat released could be determined. For sharp contrast between intact fat cells and those having completely or partially lost their content, compare Figure 1 with Figures 2, 3, 5, and 6. The fact that the wall of depleted cells was intact indicated that the fat had diffused out of the cell without structural damage. From this it would seem that the fat had left the cell in a liquid state (Figures 2, 5, and 6).

[4]Consisting usually of fat cells in single files enclosed between muscle fibers within primary muscle fiber bundles and seen in histological sections as occupying the endomysial spaces.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Dispersion of released fat. In all sections examined, any area of fat, regardless of size and location, was stained the same color (red, in case of Nile blue sulfate, orange with Sudan IV). This makes it easy to spot the fat wherever located and in whatever form it may be. It was thought, at first, that the released fat might simply flow in the interfibular spaces and cover the adjacent muscle fibers. But such was not found to be the case; instead, the fat was observed to have undergone a progressive dispersion from its source (where depletion had taken place), spreading as it proceeded and often resulting in a trail of considerable size (Figures 3, 4, and 5). With this continuous dispersion, the size of the resulting droplets became increasingly smaller until at the extremes of a dispersion field the particles were beyond microscopic resolution. For example, Figure 4 (lower left) shows the cloudy appearance of an aggregate of extremely small particles of fat when viewed with the high power objective or oil immersion lens.

Collagen and dispersion loci. In no slide studied was there any indication which might suggest a free flow of liquid fat. On the contrary, one invariably found that the fat, from the moment of its release, did not move as a continuous fluid because such a free movement was not physically possible owing to certain conditions in the immediate surroundings. Since perimysial fat islands are often surrounded by or closely associated with collagen, the movement of the released fat appeared to be significantly influenced by it. In fact, it was repeatedly observed that if dispersion occurred within a perimysium of considerable size, and if the collagen in it was only partly degraded by hydrolysis (Figures 6 and 7), the fat droplets would tend to mingle only with the degraded collagen, leaving the intact collagenous fibrils relatively free of fat. This interesting differential behavior of the intact and degraded collagen toward the dispersed fat was a substantiated observation. It was more frequently found in preparations of the *Semitendinosus* muscle (Figure 7), since its perimysia are both larger and more numerous than those in the *Longissimus dorsi*. This observation explains why

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Figure 1. Raw fresh light good ribeye, control, showing parts of two perimysial fat islands with cells intact and loaded with fat. × 20

Figure 2. Section of above sample after it was cooked, showing two partly depleted fat islands surrounded by partially hydrolyzed collagen. Three empty fat cells with distinct walls and nuclei are seen in the upper fat island, whereas more than half of the cells in the lower fat island had released their contents. × 55

Figure 3. Another cooked fresh light good ribeye, showing a typical fat dispersion center with a small perimysial fat island supplying the material. The dispersed fat followed a course approximately coinciding with that of hydrolyzed perimysial collagen, little or no fat being spread into the endomysial spaces. × 55

Figure 4. Another section of the above cooked sample, portraying part of an extended dispersion field; cells supplying the fat were located in the upper right corner of figure, not shown. Note the density of the fat droplets and their gradual decrease in size in the direction of dispersion (from right to left, and downward). × 55

Figure 5. Cooked 14-day aged heavy Prime ribeye, showing a dispersion field extending into the neighboring perimysial spaces, which separated adjacent muscle fiber bundles. Note absence of dispersed fat in any endomysial space, notwithstanding the fact that in this section the endomysial spaces and some of the perimysial spaces were about the same size. × 55

Figure 6. Cooked fresh heavy Prime ribeye, showing one large perimysium the lower half of which was filled with thoroughly mixed fat droplets and hydrolyzed collagen. The dispersed fat came from two fat islands, the one on left displaying many emptied cells with distinctly stained nuclei toward the edge of the figure. The fat island on the right contained partially emptied cells, connected directly with the dispersion field. Note that the relatively intact collagenous fibrils interposed between the two fat islands were largely free of fat. × 55

Figure 7. Cooked unaged cow round, revealing at a higher magnification a differential response of intact (right) and hydrolyzed (left) collagen toward the fat droplets in part of a dispersion field. The fat island which supplied the dispersion material is not included in the figure. Fat droplets mingled with the intact collagenous fibrils were believed to have originated, at least in part, from some of the cells in the fat island seen at the right lower corner of the figure. × 110

the released fat had no chance for continued existence in the liquid state, and partly also why fat dispersion seldom extended into the endomysial spaces.

Extent of fat dispersion. The degree of fat dispersion measurable by the length and width of a dispersion field seems to be a function of the amount of freed fat available. There have been, however, some indications that under the conditions the steaks were broiled, the degree of dispersion might and could be influenced by differences inherent in the muscle and differences in aging period. Thus, fields of dispersion were generally larger in *Longissimus dorsi* than in *Semitendinosus*, and fat seemed to disperse farther and further in fresh than in aged samples of either muscle. This latter observation may be better comprehended if one bears in mind the fact that although collagen is degraded (denatured) both during aging and cooking, the two processes are not of a similar nature.[5] Consequently, it is quite possible that in cooked aged beef, the extent of fat dispersion may not be determined simply by the amount of degraded collagen present.

It is reasonable to expect cooking temperature and time to be a likely factor in fat dispersion; but it would be presumptuous to think, without further experimentation, that both the nature and degree of the observed fat dispersion might not vary with different methods of cooking.

DISCUSSION

The one organoleptic characteristic of meat that has received the greatest amount of attention from workers in fields of food research is tenderness. This is not surprising because tenderness is something quite tangible and most appealing to the consumers. There have been various physical tests for this important meat property, but the one most used is the shear test since many workers have shown good agreement between mechanical shear test and taste panel tenderness score *(2, 7, 8, 11, 13,* and *15)*. It is widely accepted that collagen hydrolysis which occurs during cooking is responsible for meat tenderization to a very substantial degree *(5, 8, 10)*. The elastic tissue plays a relatively minor, though similar, role *(5, 8, 10, 12,* and *18)*. Muscle fibers, on the other hand, are hardened by heat probably as a result of denaturation of muscle protein *(8, 9)*, thereby counteracting the tenderizing effect achieved by softening of the connective tissues. The role played by fat in tenderness is relatively little understood. It was reported *(4)* that beef from cattle before fattening was less tender than beef from similar cattle after fattening; indicating, therefore, that fat may be a factor in improving tenderness. Other workers *(3)*, however, failed to find any significant correlation between the fat content and tenderness of cooked beef *Longissimus dorsi*. Nor was a relationship found between the amount of fat in a muscle and the shear force of both raw and cooked muscles *(3, 9,* and *14)*. Recently, two lines of work have stimulated new interest in fat. Schotte *(16)* patented a process of "artificial feeding" in which melted tallow, lard, or other edible fat is injected through the circulatory system in lean animals at the time of slaughter into the muscles to simulate "marbling," thereby to obtain tenderization generally attributable to "marbling." Our own results (to be published later) from a rather extensive study of beef quality have indicated a dual role of fat. It has been found that, whereas the total amount of fat present in a muscle is closely related to juiciness, the manner it which the fat is distributed throughout the muscle affects tenderness appreciably. This

[5] It is well known, for instance, that the enzyme collagenase is responsible for the breakdown of collagen during aging, whereas collagen hydrolysis caused by heating is not an enzymatic reaction.

function of fat distribution may be represented by the amount of surface contact between fat cells and muscle protein (either actomyosin or collagen, or both), the protein-fat interphase. It can be determined quantitatively by measuring the longest axis of each fat island, and expressing the sum of this value as the "linear" fat in a raw sample (e.g., mm. per 150 mm.^{2}). By so doing, it has been consistently shown that the tenderness score of a cooked sample correlates well with the "linear" fat content of raw. That is, the higher the "linear" fat, the more tender it becomes upon cooking. Conceivably, the beneficial effect of "marbling" may be explained on this basis.

This brings us to the question as to the role played by fat in tenderization. Can the phenomenon of fat translocation shed any light on this important question? First, it is necessary to assume that the amount of fat that can be released from a fat island depends on a mathematical ratio of diffusible surface to total fat mass; that is, the smaller the island, the larger the amount of fat released per unit volume of adipose tissue. This, then, offers a basis for understanding the observed correlation between "linear" fat and tenderness since the more "linear" fat a sample has the greater the amount of fat released for dispersion. Second, it is almost imperative to recognize the significance of the new system of degraded (hydrolyzed) collagen and dispersed fat formed during cooking. This system may be theoretically considered as an emulsion with the two substances constituting, respectively, the continuous and the discontinuous phases. The fact that only degraded collagen enters into this formation is highly suggestive of the possibility that it could actually act as a dispersing agent. Some of the physical conditions conducive to and necessary for the emulsification might be the sustained heat, the electrical charge on the fat droplets and, above all, the inherent surface properties of both of the substances involved. If so, this means cooking transforms one system of protein-fat interphase into another. Whereas in the raw muscle the contact between muscle protein (actomyosin) and fat or between collagen and fat is at a cellular level, this system becomes reduced to a lower level of organization during cooking. The importance of this transformation cannot be dismissed for the reason that our taste buds most certainly respond to this new rather than the old protein-fat system. However, one needs to be cautious that these statements of concept apply only to the specific conditions under which the steaks were broiled in the experiments. It may very well be that these results can and would be greatly altered under other cooking conditions or methods.

The foregoing considerations logically lead one to the conclusion that tenderness is something jointly determined by practically all tissue components of muscle and, therefore, the net tenderizing effect of cooking is the result of the combined action of the muscle fibers and the connective tissues, which run in opposite directions. Since the muscle fibers are toughened during cooking (*14*, *15*, and *18*),[1] the tenderization achieved by the collagen-fat system must be more than enough to offset the counteracting effect on the muscle fibers. Following this reasoning through, there

[1] Confirmed by physical tests on isolated single muscle fibers conducted in this laboratory (detailed account to be published later).

should be an ideal point reached during cooking when the maximum resultant net gain in tenderness is obtained (at point X in Diagram 1). This point is, of course, determined by many variable factors in both the cooking procedure and in the meat itself. Nevertheless, the general principle can be easily explained with the aid of a graph (Diagram 1).

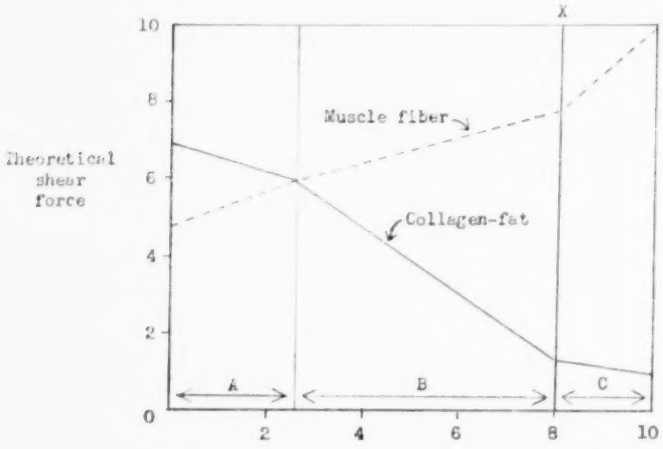

Diagram 1. Effect of cooking on tenderness.

The slopes of both the muscle fibers and collagen-fat curves are arbitrarily drawn so as to bring out the salient points in this theoretical discussion. The net tenderness at any point is expressed by the sum of the shear force of both these structures; accordingly, the ideal point (X) is reached when this sum is at a minimum. The graph incidentally also shows some other things. During period A, no significant change in tenderness occurs since the opposing effects on the muscle fibers and the collegen-fat cancel each other. During period B, there is an increasing tenderness as the tenderizing effect of the collagen-fat exceeds the hardening of muscle fibers. In period C, the net tenderness decreases owing to an accelerated toughening of the muscle fibers coupled with a slowing down of the softening of the connective tissues, such as may happen in over-cooking.

We may even go one step further in applying this principle to a more realistic consideration. In a schematic representation below (Table 1), numerical values for theoretical shear index are assigned to the tissue components of 3 beef samples, I, II, and III. The effect of cooking is indicated by the changes in these values before and after cooking, and the final tenderness score of each sample is shown inversely proportional to the sum of the combined value. For sake of clarity, these 3 samples are assumed to have equal shear rating of the raw muscle fibers, and they are, therefore, toughened to the same degree during cooking. In actual cases, however, as has been amply demonstrated *(14* and *15)*, the muscle fibers differ in this value from muscle to muscle and even from

TABLE 1

Changes in tenderness index of the tissue elements of meat during cooking

	Raw	Cooked	Samples with decreasing protein-fat interphase in raw		
			I	II	III
Muscle fiber	2		3	3	3
Collagen	7		3	4	5
Fat	1				
Total	10		6	7	8 Tenderness Score

one portion to another within the same muscle. This, of course, can be readily accommodated by assigning different shear value to them. The 3 samples, as indicated in the table, have varying amounts of "linear" fat, hence tenderized to proportionally different degrees. Elastin is omitted from this consideration because of its generally recognized minor role in tenderness *(12)*.

SUMMARY AND CONCLUSION

Observations made on a large number of frozen sections of cooked beef *Longissimus dorsi* (ribeye) and *Semitendinosus* (round) muscles stained with Sudan IV or Nile blue sulfate have disclosed a phenomenon of fat translocation and dispersion not recognized previously. Under the conditions the steaks were broiled (to an internal temperature of 150°F.), a fair amount of fat was released by diffusion from the fat cells in perimysia of all sizes. The released fat gave no evidence whatever of existence in a continuous liquid state, but showed a progressive dispersion along the path of degraded (hydrolyzed) collagen with the resulting fat droplets thoroughly mingled with the latter. Intact collagen and endomysial spaces were free of this fat. The size of the fat droplets was found to decrease from the dispersion center toward the periphery of a field, finally passing below the limits of microscopic resolution. The phenomenon has been interpreted as a process of emulsification in which the degraded collagen may function as a dispersing agent, and the heat accompanying cooking would provide, among other things, the necessary physical agitation.

Possible relations of this phenomenon to known correlations between the changes in meat effected by cooking and the organoleptic characteristic of tenderness are discussed.

Acknowledgment

The writers wish to express their sincere appreciation to Miss Eugenia Hoffert, Chief of our Home Economics Division, for preparing the broiled steaks used in this study.

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HISTOLOGICAL SURVEY OF CONDITIONS INFLUENCING TEXTURE IN POTATOES. I. EFFECTS OF HEAT TREATMENTS ON STRUCTURE

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Causes of mealiness and nonmealiness in cooked potatoes have been widely investigated. These textural qualities are important in commercial processing because cooking pretreatments frequently are employed and the final products usually are used in a cooked form. Many processors attempt to select raw potatoes according to known culinary properties. A common method is based upon differences in specific gravity. Variations in specific gravity and in starch within individual tubers, as well as between "mealy" and "waxy," have been reported *(28)*. These reflect differences in tissue composition, cellular structure, and other cell contents.

Various workers have investigated roles of moisture content, pectins, and starch as causal factors in texture *(2, 3, 9, 10, 14, 16, 17, 18, 25, 26)*. Some interpretations are contradictory, and because these and other published investigations have been reviewed elsewhere *(27, 28)*, they need not be discussed here. For the most part each study has been confined to a particular processing treatment, and consideration of variables in tissue structure has been limited. Microscopic observations on heated tubers have been either incomplete or lacking, so that a coherent picture of the relation of cellular changes to texture under different conditions is not available. The value of a more critical histological appraisal of texture problems is well illustrated by examples selected by Weier and Stocking *(27)* for several fruits and vegetables.

Histological investigations on processed potatoes were conducted extensively at this Laboratory during World War II. Some of the studies have been published *(19)*. Others were made on cellular changes in potatoes subjected to a variety of heat treatments. Because of continued interest in potato technology it seems profitable to review those studies for information pertinent to new potato products.

This first communication surveys the effects of simple heating treatments on tissue structure and elucidates mechanisms of cellular change which relate to tissue sloughing. A second report will describe microscopic distinctions of starches and accompanying cellular changes which influence texture in differently treated tubers. Additional histological observations on dehydrated products will be described in a third paper.

MATERIALS AND METHODS

Characteristics of potatoes used. These studies are confined to two varieties, White Rose from California and Russet Burbank from Idaho and Oregon. Samples of the latter included "Netted Gems" from Oregon, but this name is synonymous with Russet Burbank *(4, 24)*. Mature tubers of White Rose possess a smooth, light colored, relatively thin skin, while those of Russet Burbank have comparatively thick, russet skins

[a]Agricultural Research Service, U. S. Department of Agriculture.

with heavily reticulated surfaces. The White Rose is sometimes arbitrarily characterized as nonmealy and the Russet Burbank as mealy when cooked.

Mealiness is a characteristic of steamed or baked tubers that are described as finely granular and crumbly in both appearance and feeling when tasted. Nonmealiness includes several qualities, ranging from nearly mealy (creamy or fluffy when whipped) to waxy, sticky, or soggy qualities that may be inherent or due to culinary methods. In the absence of objective tests for all of these qualities, descriptions of texture that follow are based upon purely arbitrary and subjective appraisal considered to be adequate for histological comparisons.

Thus selection of varieties to represent textural classifications cannot be made without qualification. Among the Russet Burbank tubers used, variations in mealiness were observed in both histological and pilot-plant investigations. For example, those from Idaho tended to be more mealy when mature than mature samples from Oregon. In samples from both localities, the younger were less mealy than the mature tubers. Mature tubers of White Rose ranged from less mealy to definitely nonmealy, by comparison with the Russet Burbank, and young White Rose tubers frequently were either sticky or gummy. It is possible that these qualities are associated with starch content. Starch analyses were not made for the present study, in which the purpose was a survey of conditions related to sloughing and texture from a more general histological point of view. Thus, gross variations in tuber maturity aid in interpretation of structural changes caused by processing, because they provide a range within which histological observations are consistent.

Wide ranges of quality were obtained by use of definitely young and fully mature tubers. The younger tubers were used within 2 or 3 weeks after harvest. In general, they were slightly smaller and had thinner, more lightly colored skins than did the mature tubers *(4, 24)*. The condition of the skin is a fairly reliable criterion and can be verified by histological examination of the activity of the cork cambium, which produces the skin tissues *(1)*.

Mature tubers recently harvested (within 2 or 3 weeks) were compared with those held in cold storage up to 5 or 6 months. Some tubers also were held at room temperature for 3 or 4 weeks to alter their physiological condition as evidenced by sprouting, shrinkage, and reduced tissue turgor. Tissue turgor, as influenced by the amount of water in the cells, has been suggested by Weier and Stocking *(27)* as an important factor influencing texture; others also have considered water content to be important. Variations in turgor reflect differences in osmotic conditions (such as concentration of solutes *in toto*) in the living tissues and are thus more appropriate for histological considerations than are analyses of proximate composition.

Experimental heat treatments. Longitudinal and transverse slices one-quarter to one-half inch thick of unpeeled tubers were boiled in water to observe rates of sloughing in different tissue regions and in different tubers. Other slices were heated in steam. These experiments were repeated many times with tubers representing various conditions described above. In addition to the natural low turgor of potatoes stored at room temperature, decreased turgor also was obtained by soaking slices of fresh tubers in 2 to 5% solutions of NaCl until the tissue was limp prior to heating. Increased and abnormally high tissue turgor was obtained by soaking slices in cold water for 30 to 60 minutes before they were heated.

In other pretreatments, slices were soaked in water for 30 to 90 minutes either at 75°C.(167°F.) or at 90°C.(194°F.), then cooled to about room temperature before boiling or steaming to learn the effects of preheating upon final cellular change and texture. This resulted in some water loss but the slices so treated were not comparable with those of low turgor because soaking at these temperatures killed the tissue. The treatment provided slices, however, in which starch gelation was begun before the final heating.

Small samples of tissue were removed for microscopic examination at intervals during each boiling or steaming experiment to observe the progress of cellular changes. These samples were placed in small amounts of water on microslides, covered with a cover glass, and examined at low and medium magnifications. Particular attention was given to frequency of cell rupturing and the conditions attendant.

Histological methods. Sections were prepared from fresh tubers as well as from paraffin-embedded tissues according to standard methods *(12, 20)*. In addition, fresh

sections 500 to 1,000 μ thick were heated in water on microslides to permit observation of cellular changes *in situ*. Some were boiled and others were heated at 75°C.(167°F.) and 90°C.(194°F.) in an oven; still others were steamed over a beaker of boiling water. Observations were made at frequent intervals during treatments. Similar methods were used by others *(5, 14, 27)* to simulate conditions of some commercial processing methods. Photomicrographs were taken on fine-grain panchromatic film.

ANATOMY OF THE TUBER

The structural development of the potato tuber has been described in detail by others *(1, 11)*. Since little attention has been given in most investigations of texture to structural variation within the tuber, salient features of anatomy are reviewed here to provide orientation.

The tuber is an abruptly thickened underground stem possessing an internal tissue organization and an external arrangement of lateral organs fundamentally like that of the aërial stem of the plant. The "eyes" consist of rudimentary scale leaves and their axillary buds; these arise in a progressively congested spiral from "stem end" to "bud end" of the tuber. A corky skin is developed from cell divisions in a cork cambium as is the bark of a stem. The "stem end" is histologically older than the "bud end" and, except for an exaggerated circumferential growth, tuber growth is like that of a terminal bud of an aërial stem—by new tissue being added apically while differentiating and maturing from the basal end upwards.

Variations in cell contents in different tissue have received little attention in studies on sloughing and texture. These variables are significant. Figures 1 to 7 show the different tissues, their cell size and cell contents. Under the skin the cortical parenchyma contains smaller cells and more starch granules per unit volume than does most of the pith parenchyma further inward. Strands of vascular tissues (xylem and phloem) mark an interrupted boundary between cortex and pith; strands of internal phloem traverse the pith. In some food research literature "inner" and "outer medula" have been used for cortex and pith, respectively. In older botanical literature "pith" and "medula" are nearly synonymous. Histologically, pith and cortex are considered as "ground" or fundamental parenchyma *(8)*.

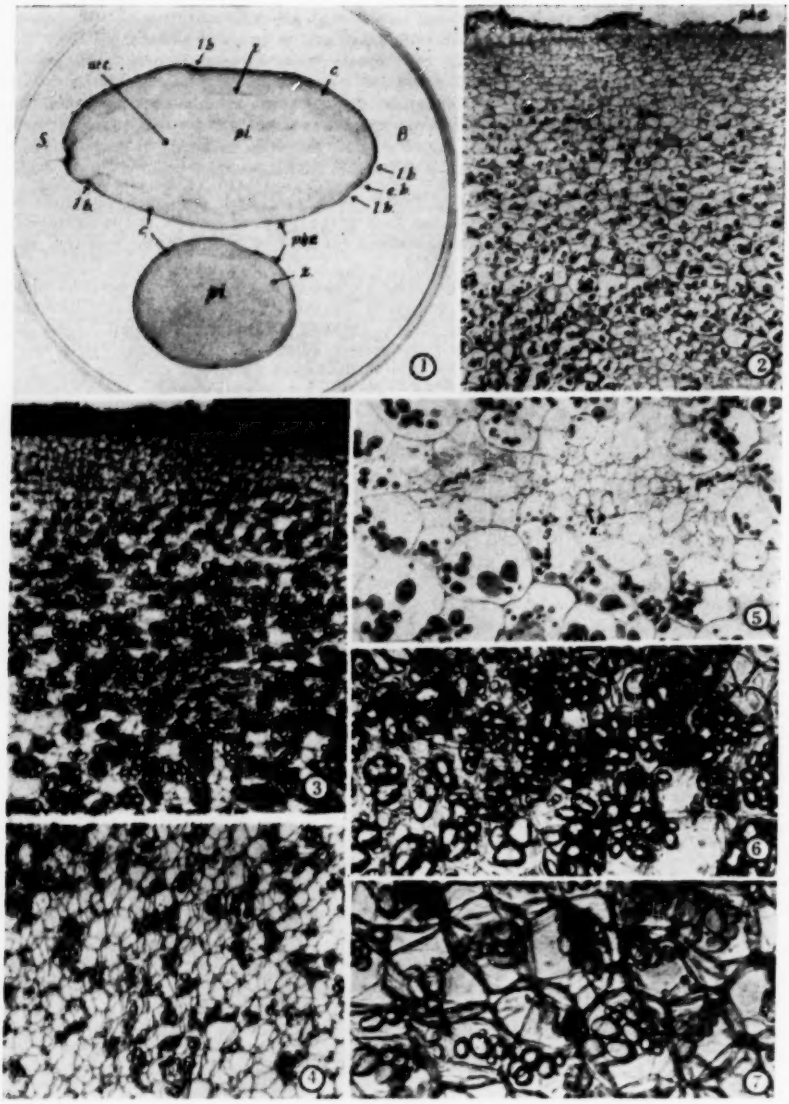
Parenchyma cells closely associated with vascular strands are small and contain small starch granules; surrounding these are large parenchyma cells with numerous, large starch granules (Figure 5). In transverse sections these areas of high starch content appear as islands between which there are other large parenchyma cells with large but fewer starch granules. The areas containing less starch radiate irregularly from the center of the pith which is also composed of large cells with less starch and is frequently called the "water core" (Figure 7). The cortical parenchyma is more uniform in cell size and starch content.

These readily visible differences in cell size and starch contents serve well to explain the variations in specific gravity within individual tubers as reported by Whittenberger and Nutting *(28)*.

Other compositional variations within individual tubers have been reported. For example, Robertson and Smith *(22)* found variations in pH within different histological areas. The greatest differences were measured during growth of young tubers and during sprout formation of mature tubers in storage. "Dormant," mature-harvest tubers showed less variation in pH. Other variations not investigated in detail for potato tubers include expected differences in cell-wall materials in the various tissues; these would be related to histological age of the cells. Changes in middle-lamella polyuronides and increased deposition of cellulosic microfibrils in the cell walls accompany maturation in all plant tissues *(11, 15, 27)*. Dastur and Agnihotri *(6)* reported considerable change in pectins during growth and storage of potatoes. Such changes are fundamental to texture qualities in both fruits and vegetables and influence changes caused by processing *(19, 21, 27)*.

OBSERVATIONS ON HEATED SLICES

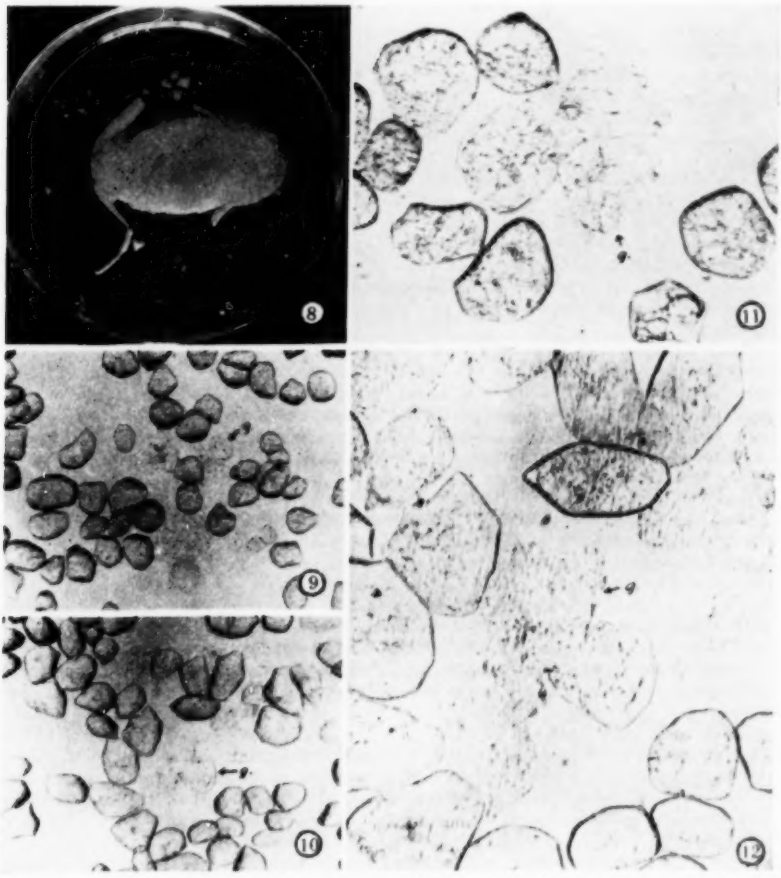
Gross changes induced by heat treatments. The progress of changes in boiled slices observed here was similar to observations reported by others on different potatoes (13). Tissue sloughing in unpeeled slices always began in the cork cambium of the skin,



Figures 1 to 7: Tuber anatomy. Figure 1. Longitudinal and transverse slices of a Russet Burbank tuber; B—bud end, c—cortex, e.b. end bud, l.b.—lateral bud, phe.—pheloderm (cork or skin), pi—pith, S—stem end, w.c.—water core, x—xylem in vascular strands. Figure 2. Section of skin and cortex; small, dark bodies in cortex are starch granules; phe.—pheloderm. × 40. Figure 3. Fresh, unstained section about 300 microns thick, showing skin and part of cortex. Black appearance of skin is due to air trapped in dead, suberized cells. × 40. Figure 4. Fresh, unstained section of part of water core area (300 microns thick). × 40. Figure 5. Prepared, stained section of vascular tissue region; ph.—phloem, s—starch, x—xylem. × 100. Figure 6. Portion of cortex shown in Figure 3. × 100. Figure 7. Portion of water core shown in Figure 4. × 100.

usually near the bud end, and progressed towards the stem end of the tuber. Sloughing spread into the cortex as the skin separated from the slice. In more advanced stages, sloughing spread into the pith (Figure 8) and tended to be most pronounced in the mid-region of the tuber. Only the extreme bud and stem ends of the slices remained intact in the most advanced stages.

Except for rate of sloughing, no marked differences were observed between mealy and nonmealy tubers. Russet Burbank tended to slough more rapidly than did White Rose when boiled. Mature tubers also sloughed more rapidly than did younger samples, some of which showed little sloughing. Replicates heated in steam did not undergo as pronounced sloughing but the cortical regions, particularly in the mid-area of both



Figures 8 to 12: Influence of heating. Figure 8. Longitudinal, unpeeled slice of Russet Burbank boiled in water and showing sloughing of cortex; bud end at left. Figures 9 and 11. Unstained separated cells of boiled, mature Russet Burbank. G—gelled starch freed from ruptured cells. Figure 9 is × 40, Figure 11 is × 100; note angular cell in Figure 11 with small amount of starch. Figures 10 and 12. Unstained separated cells of boiled, mature White Rose. G—gelled starch from ruptured cells. Figure 10 is × 40. Figure 12 is × 100; note slightly less evident reticulate appearance of gelled starch in intact cells in Figure 12 and compared with Figure 11 for Russet Burbank.

varieties, eventually sloughed when steamed for any considerable time. These variations in sloughing probably are related to differences in amount of starch in tubers, as suggested elsewhere *(38)*, as well as to starch variation within individual tubers already described.

Effect of heat on cells. Microscopic examinations showed that all sloughed tissues were composed of separated cells which were distended by their swollen starch contents. All sections of fresh tissues heated on microslides showed that starch gelation did not cause cell-wall distension until the tissues were well cooked. Until this time, pronounced swelling of the gelled starch was not evident and the cells remained angular and well coherent although their middle lamellae appeared to be appreciably weakened. All sections heated at 75° and 90°C. showed rapid gelation of starch granules, but at this temperature little or no cell-wall distension was evident and the tissues remained intact.

Microscopic examination of bud and stem-end fragments remaining intact after boiling showed that cell-wall distension was not pronounced. Cell separation with these samples could be readily induced, however, by slight pressure on the cover glass over the sample on the microslide.

Some of the separated cells of sloughed tissues from both varieties showed cell-wall rupturing and extrusion of gelled starch (Figures 9 to 12) but comparisons as to frequency of cell rupturing could not be made with any appreciable accuracy. Some increase in cell rupturing occurred during observations. Use of oil or other nonaqueous mountants and examination without fluid on the microslide imposed impractical conditions for microscopy. In addition, the small amount of material observable in one microscopic field (or several) at low magnification is infinitesimal compared with the amount of sloughed tissue per sample. Consequently, only rapid qualitative estimates on frequency of cell rupturing could be made. In making these estimates considerable caution was taken to avoid pressure on the cover glass as an additional cause of cell rupturing.

More cell rupturing was observed in all boiled than in steamed slices. Mature White Rose showed a greater tendency towards cell rupturing than did mature Russet Burbank. Slightly more rupturing and starch extrusion appeared to take place in the young than in the mature Russet Burbank but this was not consistently observed for young and mature White Rose. Separated cells from steamed samples showed less cell-wall distension and rupturing than those from boiled samples, but, when the steamed samples stood for several minutes in water on the microslide, increased wall distension and cell rupturing occurred.

Ruptured cells ranged from 2 or 3 to about 40% of cells per field at 60× magnification. Cells appearing intact at this magnification showed, at 1,000× (oil immersion), minute blobs of gelled starch extruded where wall rupturing was not evident. Apparently these tiny blobs had been forced through primary wall pits in the cellulose matrix. Such pits are characteristic of many parenchymatous plant tissues and consist of minute, rounded thin areas in the cellulosic matrix which have been shown by electron microscopy to contain fewer cellulose microfibrils than do surrounding wall regions *(8, 15)*.

Other factors related to cell rupturing. Particular attention was devoted to various conditions influencing cell rupturing and their possible relation to texture. In most instances the experiments and observations provided no conclusive evidence. Recently harvested and cold-storage tubers showed no appreciable difference. Consistent relation of cell rupturing to texture was obtained with only two specific conditions: (a) effects of warm soaking treatment prior to final heating and (b) variations in fresh tissue turgor.

Slices soaked for different periods at 75°C. and then steamed or boiled showed no marked differences in texture according to time of pretreatment or final heat treatment. All tended to be leathery when subsequently cooled and leatheriness was more pronounced with increased time of pretreatment. Slices soaked at 90°C. prior to steaming or boiling were similar to cooked potatoes of "waxy" texture when subsequently cooled. These showed some variation with time of soaking; those soaked for 30 to 45 minutes tended to be slightly leathery when cooled, and waxiness was more pronounced in White Rose than in Russet Burbank samples soaked for longer periods. Some White Rose samples soaked 90 minutes at 90°C. were gummy or sticky when subsequently boiled.

No sloughing was observed when the slices soaked at 75°C. were either boiled or steamed. Slight sloughing occurred with White Rose samples boiled after soaking 90 minutes at 90°C. Only a few ruptured cells were observed after final heating of soaked

slices; these were nearly always found in slices soaked 90 minutes at 90°C. and mainly in White Rose samples which were sticky or gummy. Further details concerning these pretreatments will be presented in the second part of this review with reference to observable differences in the starches *in situ*.

All slices soaked in cold water to induce high tissue turgor showed definite increases in cell rupturing when boiled or steamed. All mature tubers stored at room temperature until sprouting and low turgor were evident showed definitely less cell rupturing when boiled or steamed than did samples of "normal" turgor. Low turgor White Rose tubers, when boiled or steamed, were nearly as mealy as those of Russet Burbank and showed very little cell rupturing. All slices soaked in salt solutions to reduce turgor were similar in texture to other low-turgor samples when steamed or boiled although the difference, by comparison with controls, was not as striking as those just described.

Increased turgor of originally low-turgor (sprouting) tubers, by cold-water soak, resulted in increased cell rupturing and sloughing when boiled and steamed. Mature White Rose tubers of this sort showed an appreciable amount of cell rupturing when steamed and their texture was gummy. Comparable slices of Russet Burbank also showed an increase in cell rupturing and their texture was less mealy than that of controls.

Among the several combinations of young and mature tubers, in which changes in turgor were induced, considerable gradations in sloughing rates, textural qualities, and cell rupturing were obtained. In general, rates of sloughing and frequency of cell rupturing increased proportionately with increased turgor. Increased sogginess in boiled slices and increased gumminess in steamed slices of White Rose were associated with frequency of ruptured cells. Reduced mealiness, even to the point of stickiness, was associated with increases in cell rupturing in Russet Burbank. These observations are in agreement with the view expressed by Weier and Stocking *(27)* concerning the influence of tissue turgor and cell rupturing upon texture. It should be mentioned, however, that storage potatoes of low turgor also have undergone changes in sugar and starch contents which also may be correlated with the textural qualities produced by heating.

DISCUSSION

The results of this present survey provide some distinction between mechanisms of cellular change and textural qualities in different potatoes. The distinction is not always clear, however, because changes such as sloughing, cell rupturing, and starch gelation are interdependent in their influence upon texture. In addition, tuber conditions and pretreatments modify that influence and alter the magnitude of cellular changes that are observable. It would be misleading, therefore, to single out any one factor thus far described as a direct cause of a given textural quality. Conditions other than those investigated here may also modify the histological phenomena which result with processing treatments. For example, harvesting practices in different localities, such as early and late harvest, and aging in the field, are known to influence tuber quality. Histological investigations on the effect of such practices on texture in processed potatoes should be pertinent.

It seems worth-while to compare effects of heating on potatoes with those on other fruits and vegetables in order to provide a more complete histological elucidation of textural qualities. Visible effects of processing (heating, drying, and freezing) upon intercellular gases, cell constituents, cell walls, and middle-lamella materials have been described for several fruits and vegetables *(5, 7, 21, 27)*. In summary, intercellular gases expand and are forced out by heating and drying; cooking treatments dissolve or at least weaken the middle lamellae between cell walls and coagulate the protoplasmic contents of the cells. Starch, if present, is gelled by mild heating, and cooking causes changes in some of the cell-wall materials.

An inference is sometimes encountered that cellulose walls are disintegrated as a result of ordinary cooking. Cell-wall discontinuity has been illustrated for cooked carrots and parsnips *(23)*, but critical histological evaluation of such results suggests that the discontinuity may be due more likely to artifacts of histological technique. Dehydration in alcohol, treatment with xylene or other paraffin solvent, etc., result in very brittle and sometimes distorted tissue conditions. Paraffin compression, during microtomy of the embedded samples on a rotary microtome, causes wrinkling and cell-wall fracturing in the sections unless softening treatments are used prior to sectioning *(20)*.

Changes in cellulose walls as a result of cooking (c.f. Day, *1*) can be better understood when variations in cell-wall composition in different tissues are considered. Substances other than pectic (lignins, suberins, cutins, and proteins) may be present in the middle lamellae and within the cellulosic matrix *(8, 27)*. The effects of processing on these may vary considerably and thus influence degree of cell separation. That the cellulosic matrix is composed of microfibrils in various degrees of orientation has been well demonstrated by the polarizing microscope, X-ray, and electron microscopy *(8, 12, 15, 27)*. These structural conditions influence elasticity and strength in plant cell walls.

The elasticity and resistance of parenchyma cell walls to changes in internal pressures and heating treatments have been well illustrated by studies on apples *(21)* and other fruits and vegetables *(5, 7, 27)*. The cellulosic matrix may be weakened because of removal of encrusting substances, but sufficient hydrolysis to cause discontinuity of the wall by ordinary cooking seems chemically improbable. On the other hand, effects of heat upon cell contents, such as the swelling of gelled starch in potato cells, can cause cell rupturing. Upon gelation with cooking treatment, the starch granules become so swollen that their individuality within the cells is not readily demonstrated. The internal pressures of potato cells fully packed with starch granules must be considerable when the granules gel and swell with cooking treatments. Weak cell-wall areas, such as the simple primary wall pits, provide logical points for the beginning of cell rupturing.

The zonal pattern of sloughing in longitudinal slices of potatoes has some bearing upon commercial peeling methods. Softening and sloughing of outer (cortex) tissues may be accentuated when a lye-scald is used because of the effect on the middle lamellae *(27)*. With other methods of peeling by heat, starch gelation may occur to some depth beneath the removed skin. This area of gelled starch can result in a toughened case when the peeled potatoes are stored because of changes in the gelled starch.

In discussing mechanisms of sloughing, Whittenberger and Nutting *(28)* state that "In waxy or soggy tissues the cells show less rounding and remain firmly stuck together. Cells separate (they seldom rupture) because of the failure of intercellular cement, which is composed of pectic compounds." Although the inference that sloughing is not caused by cell rupturing is correct, the observation on cell swelling appears to be at variance with the present observations. Frequency of cell rupturing sometimes may be high in cooked potato tissues; both cell swelling and cell

rupturing here were found to be more pronounced in nonmealy than in mealy potatoes. It seems likely, however, that the discrepancies between these observations may be due to differences in degree of heat treatment and in varieties investigated.

Histologically, tissue softening, starch gelation and swelling, cell separation, and cell-wall distension and rupturing are interdependent phases of the structural changes caused by cooking potatoes. This is essentially in agreement with the conclusions of Personius and Sharp *(16, 17, 18)* that neither failure of cell cohesion nor starch gelation, alone or in combination, is a causal factor of texture in potatoes, but rather both are mechanisms of structural change.

The differences in cell swelling and rupturing between White Rose and Russet Burbank, as observed here, suggest that certain properties of their gelled starches may influence their texture. Some of the properties of starch gels from other potatoes have been investigated *(3)* and the results lend support to this view.

SUMMARY

Histological observations on experimentally heated White Rose and Russet Burbank potatoes have demonstrated that tissue sloughing (cell separation) is not a direct distinction between mealy and nonmealy cooked potatoes. Cell separation, due to weakening of the adhesive properties of the middle lamellae, is aided by the swelling of gelled starch within the cells so that their walls are distended and pushed apart.

Cell rupturing also is caused by swelling of the gelled starch and appears to begin in the weaker primary pit regions of the cell walls. Sloughing was less pronounced in White Rose than in Russet Burbank but cell rupturing was more pronounced in the White Rose. Both sloughing and cell rupturing showed proportional increases when increase in original tissue turgor of the samples was induced prior to heating.

Escape of gelled starch from ruptured cells in cooked tissues caused a sticky or gummy texture. In cooked samples of waxy texture, cell swelling did not progress sufficiently to cause sloughing. Mealy cooked tissues showed ready cell separation but not enough cell swelling to result in much cell rupturing and escape of gelled starch.

The progress of sloughing in heated slices of unpeeled tubers demonstrated a relation of cell separation to histological variations within the tuber. The tendency for the cortical region of the tuber to slough readily, and the gelation of starch by heat, have bearing on problems of case hardening and external sloughing encountered with commercial methods of peeling by heat and by lye-scald.

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HISTOLOGICAL SURVEY OF CONDITIONS INFLUENCING TEXTURE IN POTATOES. II. OBSERVATIONS ON STARCH IN TREATED CELLS

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In the first paper of this series *(9)* it was demonstrated that starch gelation, cell separation, and cell rupturing are interdependent changes that occur when potatoes are boiled or steamed. These constitute mechanisms of tissue alteration and are not primary causes of different textural qualities in cooked potatoes, because all three occur in both mealy and nonmealy tubers and vary according to heat treatments and other variables. Observable differences in the starches, *in situ*, of mealy and nonmealy tubers are described in this second communication.

MATERIALS AND METHODS

White Rose and Russet Burbank potatoes of extreme variation in maturity, as described previously *(9)*, were used. Chemical simulation of cooking was accomplished by essentially the same methods reported by Personius and Sharp *(7, 8)*. That is, chloral hydrate solutions were used to cause starch gelation. Cell separation in sections of fresh tissues was induced by treatments with 0.5 to 1.0% solutions of ammonium oxalate, oxalic acid, and hydrochloric acid for an hour or more at 80°C.(176°F.). These latter treatments also caused gelation of most of the starch granules in the cells but the starch so gelled did not undergo as much hydration and swelling as occurs with ordinary cooking *(9)*.

A dilute aqueous solution of iodine and potassium iodide was used for staining starch. Lugol's iodine (6 g. KI and 4 g. I_2 in 100 ml. H_2O) was diluted to about 0.1% total iodine. Care was taken to keep the amount of solution applied to microslide preparations approximately the same for comparisons of staining of different samples.

The histological investigations on experimentally treated sections were repeated on a gross scale, with quarter-inch thick slices from each variety. Some slices were boiled until sloughing was pronounced; others were steamed 30 minutes or longer. A third lot was soaked at 80°C.(176°F.) in the pectin solubilizing reagents to induce cell separation. The three lots of slices were prepared by serial replication from several tubers to insure uniform sampling. After the treatments, the tissue weights and media were equalized with added water in which the samples were stirred until some cell separation was evident in all of them. They were then filtered. Both filtrates and tissue residues were stained with the diluted iodine solution. Other experimental methods previously described *(9)* will be referred to later.

OBSERVATIONS

Gelation of starch. Gelation of potato starch, by heat in water, begins at about 60°C.(140°F.), and at 65°C.(149°F.) most of the granules have swollen sufficiently so that their optical birefringence, as shown by the polarizing microscope, is no longer evident *(11)*. Swelling of granules with continued heating and at higher temperatures progresses until the individual granules are not readily distinguishable. Treatment with 40% chloral hydrate solution at room temperature or lower also causes starch gelation.

The relation of starch gelation to cell swelling, cell rupturing, and texture in slices of White Rose and Russet Burbank subjected to different heating treatments are com-

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pared in Tables 1 and 2. These results include slices soaked at 75°C.(167°F.) in water, then cooled before final heating as previously described *(9)*. Treatment with chloral hydrate, as applied to sections of the tubers, is not included in the tables. In general, however, results and observations obtained with other experimentally treated sections were the same as those listed for treated slices in Tables 1 and 2.

Chloral hydrate treatment did not cause as much swelling of gelled starch and consequent cell wall distension in tissue sections as did the various heat treatments. Thus, cell rupturing was not observed in chemically treated sections unless they were left soaking in chloral hydrate for long periods. Other chemical treatments to induce cell separation likewise caused no cell rupturing at the temperature used.

Although cell rupturing and cell separation sometimes appear to be correlated with textural qualities, it is evident that other factors are involved. The importance of starch gel escaped from ruptured cells has been mentioned previously *(9, 11)* and is shown in Table 1 to be correlated with some of the differences in textural qualities of both young and mature White Rose and Russet Burbank tubers.

Observable differences in starches of tubers. When thick sections (300 to 500 μ) of tubers were carefully heated by boiling or steaming on microslides and stained with dilute iodine, differences in starch staining were observed. Sections of White Rose always showed a typical "amyloid" blue[b] in the solution external to the cells when examined microscopically. Only very faint, if any, blue color with iodine was observed in the external solutions of sections of the Russet Burbank. The blue coloring in the extra-cellular solution was slightly more evident with heated sections of younger than those of the mature Russet Burbank. Intense amyloid blue in the extra-cellular solu

TABLE 1

Comparisons of cell separation, cell rupturing, and staining of starch in heated slices of potato tubers and their relation to final texture qualities

Treatments and raw materials	Cell separation	Cell rupturing	Iodine color[1]		Texture of tissue after final heating
			Gelled starch	External solution of filtrates (amyloid blue)	
Boiled					
Mature White Rose	Slough in water	++++	Reddish to blue-purple	+++	Slightly mealy soggy
Young White Rose	Slough in water	++++	Reddish to blue-purple	++++	Soggy or sticky
Mature Russet Burbank	Slough in water	++	Blue-black	Faint, if any	Mealy when sloughing not severe
Young Russet Burbank	Slough in water	+++	Blue-black	+	Slightly mealy to soggy
Steamed					
Mature White Rose	Readily obtained on microslide	++	Reddish purple	+++	Slightly mealy
Young White Rose	Readily obtained on microslide	++	Reddish to blue purple	++++	Generally sticky or gummy, sometimes waxy
Mature Russet Burbank	Slight sloughing	+	Blue-black	Generally none	Mealy
Young Russet Burbank	Readily obtained on microslide	+	Blue-black or dark purple	Faint	Slightly mealy to waxy, sometimes sticky

[1] With dilute aqueous solution of iodine and potassium iodide (ca. 0.1% total iodine).

TABLE 2

Comparisons of cel separation, cell rupturing, and staining of starch in slices of soaked and chemically treated tubers

Treatments and raw materials	Cell separation	Cell rupturing	Iodine color[1]		Texture of tissue after final heating
			Gelled starch	External solutions or filtrates	
Boiled after soaking at 75°C. 1 hr.					
Mature White Rose	Readily obtained on microslide	Slight	Blue purple to black	++++	Rubbery, slightly sticky when slice is broken
Young White Rose	Readily obtained on microslide	Slight to none	Reddish purple	++++	Rubbery, slices sticky to gummy when broken
Mature Russet Burbank	Readily obtained on microslide	None	Blue-black	Faint, if any	Leathery, granular when slice is broken
Young Russet Burbank	Readily obtained on microslide	None	Blue-black	Faint	Rubbery, granular to waxy when slice is broken
Soaked 1 hour at 80°C. in 0.5% solutions of pectic solvents					
Mature White Rose	Slight, by stirring	None	Blue-purple	+	No final heating
Young White Rose	Slight, by stirring	None	Blue-purple	++	No final heating
Mature Russet Burbank	Slight, by stirring	None	Blue black	None	No final heating
Young White Burbank	Slight, by stirring	None	Blue-black	None	No final heating

[1] With dilute aqueous solution of iodine and potassium iodide (ca. 0.1% total iodine).

tions was obtained with sections of both young and mature White Rose heated until cell swelling and rupturing were pronounced.

In most instances a stereoscopic microscope permitting magnifications of 10× to 80× provided a better means of examining the stained sections than did a compound microscope because an opaque, white background could be used with the former. Thus it was possible to observe very faint amyloid blue with some treated sections and to distinguish differences in intensity of color of simultaneous comparisons of two preparations at low magnification. These distinctions are listed in Tables 1 and 2.

The same differences in staining of starch were obtained when the cells of the treated sections were deliberately crushed to free the gelled starch. The amyloid blue staining of soluble starch in external solutions could always be distinguished from the staining of the gelled starch masses, whether the latter were in intact cells or freed from rupture cells (Tables 1 and 2). The freed gelled starch did not readily wash away

[5] "Amyloid" blue refers to the color obtained with the histological test for cellulose in which the cellulose is hydrolyzed with strong acid and then stained with iodine. In the absence of soluble starch, this has been considered specific for cellulose. In the staining for starch, however, I_2 KI Solution is used alone. Iodine staining of cellulosic walls, without hydrolysis, results in yellow to orange-red colors, depending upon cell wall encrusting materials present (*10*).

when the iodine solution was carefully replaced with water; the blue-staining soluble starch was readily removed by this treatment. The gelled starch in treated sections of Russet Burbank nearly always stained a deep blue-black, particularly in the intact cells. A visibly lower intensity of staining in the gelled starch in sections of White Rose gave colors ranging from reddish to bluish purple; the red shades were more characteristic of the freed gelled starch masses.

Unstained preparations examined with the compound microscope at 100× to 250× revealed that the gelled starch in intact cells of Russet Burbank sections appeared to be more reticulate than that in the heated sections of White Rose tubers. Longree *(5)* also observed some cellular differences between cooked and frozen mealy and nonmealy tubers. She used "reticulation," however, to describe the angular appearance of cells, which was similar to the fresh, unswollen condition in possessing 10 to 14 or more flat wall facets. In this sense, pronounced reticulation of cells was associated with "cottoniness" of texture and cell shrinkage in cooked, frozen tissues.

Reticulation is used in the present studies solely to describe the gelled starch. It appeared to be an optical condition which demonstrated differences in the starch contents of the intact cells. The swollen granules are greatly entangled when confined within the cell walls. Various other cell contents, mainly protoplasmic materials, are trapped in the form of minute strands between the gelled granules to give the gelled mass the appearance of an irregular reticulum. The presence of additional substances of a refractive index close to that of gelled starch, as might be the situation with soluble starch, tended to mask the fine reticulum of entrapped protoplasm. For example, when stained with iodine, a blue color of soluble starch in intact, heated White Rose cells could be washed out by carefully replacing the iodine solution with water. When this was done, the gelled starch in such cells then showed a more evident reticulation.

Comparisons of cell separation and starch in heated and chemically treated tissues. In comparing cell rupturing and staining of soluble starch in the extra-cellular solutions of boiled and steamed tuber slices it can be seen in Table 1 that decreases in mealiness are associated with both increases in cell rupturing and intensity of amyloid blue in the external solutions. In general, mature White Rose were less mealy than mature Russet Burbank and the younger tubers of each variety displayed more nonmealiness than the mature. In each instance, the most pronounced nonmealiness (soggy or sticky) was associated with the most intense amyloid blue (with iodine) in the external solutions.

The results of chemical treatments to induce cell separation are listed in Table 2 where they are compared with the effects of soaking at 75°C. prior to final heating. It is interesting to note that White Rose samples soaked at 80°C. in pectic solvents showed some staining of the external solutions without further heating while the Russet Burbank did not. Although the different reagents produced different degrees of cell separation (upon stirring after treatment), effects on starch contents by these reagents at this temperature were essentially the same and were comparable to soaking in water at 75° or 90°C. without further heating.

Pronounced alterations of final cellular changes and texture, after boiling or steaming, were observed for both varieties when slices were soaked in water at 75° and 90°C. In all samples so treated starch gelation occurred before the final heat treatment and the slices generally remained intact after steaming. Those soaked at 75° (Table 2) were leathery or rubbery when cooled after final heating. Slices of mature Russet Burbank soaked at 75° were tougher and more leathery after final heating than those soaked at 90°C. Both soaking treatments resulted in tougher textural qualities in the Russet Burbank than in the White Rose samples. White Rose slices soaked at 90°C. had a more waxy texture when steamed, but were sticky or gummy when broken. Steamed slices of mature Russet Burbank tubers, heated and cooled after soaking, tended to be granular when broken. Softer textural qualities resulted when the soaked slices were boiled, particularly with slices of each variety soaked at 90°C.

Cell separation could be obtained on the microslide with all soaked slices when samples were examined microscopically after boiling or steaming; it was most readily obtained with the boiled slices. Except for mature White Rose, no cell rupturing was observed in any of the slices soaked at 75°, and then boiled or steamed, unless the samples remained for several minutes in water on the microslide. Cell wall distension in these micromounts was slight; nearly all cells appeared angular. When stained with dilute iodine, these micromounts of White Rose often showed even more intense amyloid

blue in the extra-cellular solutions than was observed for those merely steamed or boiled. Only faint or doubtful blue color was observed with comparable micromounts of Russet Burbank samples.

In White Rose samples soaked at 90°C. for an hour, cooled, and then steamed or boiled, cell separation and cell rupturing were less pronounced than with slices merely boiled or steamed. Staining in the extra-cellular solutions, however, appeared to be more intense in the pretreated slices; this was true of both micromounts and filtrates. Stickiness was more pronounced with slices soaked at 90°C. than those soaked at 75°C. and then steamed or boiled. The textural characteristics of mature Russet Burbank slices soaked at 90°C. and then steamed or boiled were intermediate between those of the controls and those soaked at 75°C. before final heating. In all samples of both varieties at either temperature in water, the differences in final texture between young and mature tubers were less than those listed in Table 1, which serve here as characteristic of control samples.

The texture of the slices soaked at either 75° or 90°C. tended to be similar both before and after final heat treatments and cooling to room temperature, except for White Rose slices soaked at 90°C. In other words, leatheriness or other toughness of texture in the soaked slices returned when they were cooled after steaming or boiling. This was most evident with slices soaked at 75°C. White Rose slices soaked at 90°C., however, underwent further, irreversible changes in texture when steamed or boiled.

DISCUSSION

The differences in cell swelling and cell rupturing, as influenced by the gelled starch, and differences in staining of soluble starch in the extracellular solutions and filtrates provide histological distinctions between the starches of White Rose and Russet Burbank potatoes. These distinctions, consistently obtained under different experimental conditions, suggest that the properties of the gelled starch, *in situ*, are largely responsible for the textural qualities of these potatoes. These differences may be due to chemical or physical variation in the crystalline matrices of the starch granules of the raw tissue, which could influence hydration of the gelled starch when the tissue is heated. Degree of hydration is known to influence colors produced when starches and cellulose fibers are stained with iodine *(10)*.

Different heat treatments long have been known to alter the properties of starch gels *(4, 11, 12)* which, presumably, would influence texture in cooked starchy vegetables. Barmore *(2)* measured rates of tissue softening in different potatoes, in attempting to study properties of the gelled starch *in situ*, and found variation according to time of treatment. His conclusion that the physical properties of the starches were altered is in agreement with the observations made here.

Barham and coworkers *(3)* found correlation between variety and starch gel viscosity with Kansas potatoes and observed some interesting differences in the gelation temperatures of the starch granules. In other published investigations *(3)* gels from the starches of mealy tubers have been reported to have less rigidity than those from nonmealy tubers. In this latter instance, however, the results appear to be the reverse of the present observations on the starches, *in situ*, of White Rose and Russet Burbank potatoes, in which greater hydration of the gelled starch appears to cause cell rupturing and nonmealiness. It is difficult, and possibly misleading here, to transpose results obtained with extracted starches for explaining textural qualities which are influenced by the gelled starch *in situ*, and which may also depend upon other variables. How degree of

hydration influences rigidity of gelled starch within cooked tissue remains largely conjectural.

That there are wide differences in size, number, and probably in age of the starch granules in the cells of the fresh tuber can be readily seen *(9)*. These differences may be associated with variations in composition of either the starch granules or of other cellular contents which could influence their gelling properties. For example, Meyer and Heinrich *(6)* found wide variation in amylose and amylopectin in the starches from different parts of the potato plant. Similar differences may exist between different tuber tissues, young and mature tubers, or between varieties. Further investigations are required to learn their magnitude and influence upon textural qualities.

The importance of any particular histological variable in texture quality in potatoes depends upon the influence of other factors such as storage conditions, processing treatments, and physiological conditions of the tubers. The crux of understanding texture problems, then, lies in a more comprehensive knowledge of how each separate condition of structural change influences subsequent changes and final quality. In the absence of more practical analytical methods, simple heating treatments and microscopic evaluation of the changes produced may be useful for testing potatoes for their texture when processed.

SUMMARY

Histological examinations made on the gelled starch contents in the cells of heated and chemically treated potato tissues have revealed differences in the iodine staining and optical properties of starches in mealy and nonmealy potato. Some differences in staining of gelled starch in intact cells, as well as that extruded from ruptured cells, suggest differences in the hydration properties of the gelled starch of different potatoes.

Heated White Rose tissues consistently showed more intense staining of soluble starch (amyloid blue with iodine) in extra-cellular solutions than did heated tissues of Russet Burbank. This difference was evident even when little or no cell rupturing had occurred to free the gelled starch and it was consistently correlated with nonmealy and mealy textural qualities, respectively.

The histological demonstrations reported here, and in the previous paper, support the view that the properties of the gelled starch in cooked tissues are significant to texture in potatoes. The influence of gelled starch, however, can be modified by degree of heat treatment, available water, and other conditions; thus no single factor alone may be the cause of textural quality. Simple heating experiments appear to be reliable means of testing potatoes for their texture.

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HISTOLOGICAL SURVEY OF CONDITIONS INFLUENCING TEXTURE IN POTATOES. III. STRUCTURE AND TEXTURE IN DEHYDRATED POTATOES

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During World War II many difficulties were encountered in the commercial production of dehydrated potato cubes. The most widely used process consisted of hot-air drying in a tunnel or in a cabinet after a hot-water or steam scald to inactivate enzymes. Usually the products were in the form of half cubes which dried to a brittle, shrunken, slightly amber and translucent condition. Only certain varieties appeared to be suitable *(1, 2, 3)*.

Earlier histological investigations *(13)* describe the cellular changes that resulted from this sort of processing. Since then other forms of commercially dried potato products have been developed. It seems desirable, therefore, to re-examine previously reported observations on dehydrated potatoes for information which may have bearing upon the mechanisms of cellular changes caused by processing, and the influence of such changes upon textural qualities.

In this third report brief comparisons are made of dried potato products processed by different methods. Since any consideration of histological changes caused by dehydration must necessarily include an understanding of the effects of pretreatments, such as scalding, some of the conclusions reported below are based upon previously established information on effects of heat upon tissue structure and cellular contents *(14, 15)*.

MATERIALS AND METHODS

Many of the observations here were made on commercially manufactured products as well as on samples produced either in pilot plant or laboratory-scale operations at this Laboratory. Microscopic examinations of these products in both the dried and the rehydrated states were accomplished by methods already described *(14, 15)*. Because most of the experimental treatments used here were for purposes of demonstration, they will be briefly described only where pertinent to the following observations.

INFLUENCE OF BLANCHING TIME ON DEHYDRATED POTATO CUBES

"Popcorn" effect. One of the phenomena encountered with hot air drying of blanched potato cubes was the occurrence of porous, opaque areas in otherwise translucent pieces. This was described as the "popcorn" effect by Campbell *et al. (3)*, who found that it was related to duration of blanching and was more prevalent in overly blanched cubes. Russet Burbank tubers from Idaho showed more "popcorn" areas (up to 82% of total pieces) than did Russet Burbank tubers from the Klamath, Oregon, area (up to 11% of total pieces) but only 3 lots of potatoes of one season were investigated as to percentage of "popcorn." These opaque areas rehydrated very rapidly so that uniformity was lacking in culinary use.

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In dehydrated cubes produced from the Idaho potatoes, as stated by Campbell *et al. (3)*, pronounced development of opaque areas appeared in those blanched 8 minutes prior to drying (Figures 1 and 2). Cubes processed from the Klamath, Oregon, potatoes showed little or no opacity in samples blanched 8 minutes, but opacity increased when the cubes were blanched more than 8 minutes. In both lots of potatoes, blanching for only 2 to 4 minutes largely eliminated this defect.

Microscopic examinations revealed no evidence of broken cells in the opaque areas. The presence of many minute air pockets in these areas suggested that the opaque porosity resulted from a partial cell separation and expansion of gases when the tissues were dried in hot air. Fragments of vascular tissues were frequently observed in the porous areas and this suggested that opacity might be more pronounced in cubes from certain tissues within the tuber.

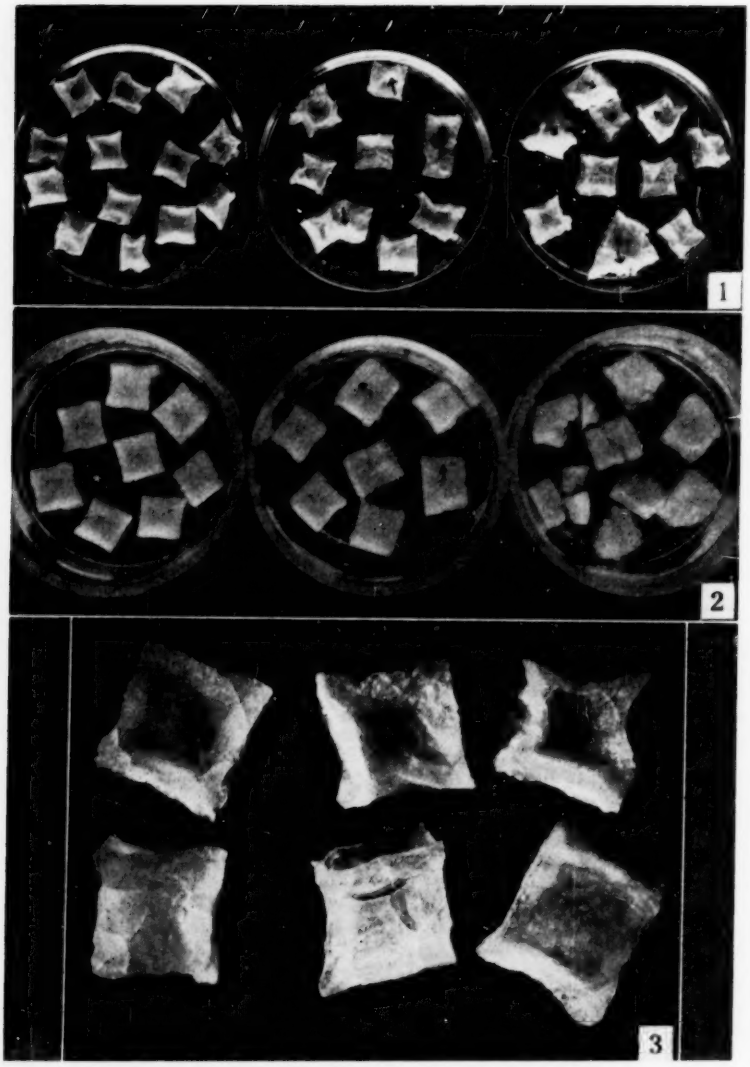
In order to verify the possible influence of variation within individual tubers on the development of opacity just described, longitudinal, unpeeled slices one-fourth inch thick were blanched 2, 4, 6, and 8 minutes and then dehydrated in a tunnel drier. Dried slices blanched only 2 minutes showed little or no opacity and that which infrequently occurred was always in the cortex immediately underlying the skin. In slices blanched for longer intervals opacity increased with increase in time of blanch. Moreover, the development of the porous opaque areas occurred in a zonal pattern which paralleled that previously described for tissue sloughing in boiled slices *(14)*, i.e., first along the cortex and then inward to the pith with increased time of blanch. Pronounced opacity developed throughout most of the slices of Russet Burbank potatoes from Idaho which had been blanched 8 minutes. Development of porosity was less pronounced in samples prepared from Oregon potatoes but essentially the same regional sequence within tuber zones prevailed.

Influence of pectic solvents and precipitates on development of porosity. Other lots of tuber slices prepared from Russet Burbank potatoes (from each location) were treated with pectic solvents and precipitates to further demonstrate the role of middle-lamella materials in development of the "popcorn" effect. This was accomplished by soaking the longitudinal slices for periods of 10 to 20 minutes in 1% solutions of ammonium oxalate, oxalic acid, and hydrochloric acid, separately, at about 40°C.(104°F.) for weakening the cementing properties of the middle lamellae prior to blanching and dehydration. Strengthening of the middle lamellae was accomplished by similar soaking treatments in 0.5% calcium chloride solutions.

The general effects of the pectin-solubilizing treatments showed no consistent correlation of porosity with potatoes from the two localities, but many slices blanched only 2 minutes after treatment showed an appreciable development of porous areas upon dehydration. All slices treated with calcium chloride solutions showed a marked decrease in the "popcorn" effect when dehydrated and only the most susceptible regions (cortex) of the slices developed porous areas in samples blanched for 8 minutes. These results, and the correlation of opacity with zonal pattern of sloughing, demonstrate the relationship of the "popcorn" effect to the relative ease of cell separation as influenced by middle lamella materials. It seems likely that the tuber areas of high starch contents (as in the cortex) also are correlated with "popcorn" effect because of greater swelling of gelled starch when the tissues are heated. Such swelling, then, contributes to ready cell displacement and cell separation.

Fracturing in dehydrated potato cubes. Another characteristic observed generally to be coincidental with the "popcorn" effect was the presence of minute fractures in dehydrated cubes that had been blanched for 4 minutes or longer (Figures 1, 2, and 3). The fractures usually did not become evident until the final stages of dehydration and sometimes not until several hours after drying had been completed. Cubes having this defect readily fragmented when rehydrated either in hot or in cold water and also appeared to rehydrate more slowly than did cubes in which fracturing was not evident.

Microscopic examinations of partly rehydrated samples showing fracturing revealed that the fracture lines cut through cell walls. The gelled starch contents of the fractured cells were freed upon complete rehydration. Freeing of gelled starch in this manner, however, did not appear to alter the textural qualities of the reconstituted product, as did freed gelled starch in boiled and steamed potatoes *(14)*, in samples prepared only from mealy Russet Burbank tubers. Some samples prepared from less mealy tubers, which showed pronounced fracturing when dehydrated, were slightly gummy when reconstituted.



Figures 1-3. Dehydrated potato cubes from Russet Burbank tubers. Figure 1—Cubers dehydrated after different blanching times; left to right, two, four, and eight minutes respectively. Note minute fracture lines in cubes of middle and right samples. Small opaque areas (arrows) do not show readily in photos because of black and white contrast. Figure 2—Rehydrated cubes replicate to those above in Figure 1; rehydration with warm water soak for 30 minutes. Figure 3—Enlargement (ca. 2½ ×) of dehydrated cubes of Russet Burbank blanched 8 minutes and showing fracture lines.

It was thought that the fracturing might indicate some change in physical properties of the gelled starch which resulted with conditions of blanching and dehydration. This was briefly investigated with gels of different concentrations prepared from potato starch by heating the starch in water at nearly boiling temperatures until samples examined with the polarizing microscope revealed no granules showing optical birefringence. Typical pastes were readily formed with low concentrations of starch in water but a translucent paste was not easily obtained with concentrations of starch approaching that of the fresh tissue (about 10 to 12%). One sample of each paste was dried to brittleness in an oven at about 75° to 80°C.(167° to 175°F.); corresponding samples were heated to near boiling for 10 to 12 minutes prior to drying.

After drying, those gels which had an original starch concentration approaching the condition in fresh tissues, and had received additional heating prior to drying, showed minute fractures comparable with those in the potato cubes. No fractures were observed in the other dried gels. Rate of rehydration of the fractured gels was slow by comparison with those not fractured.

Although no other studies were made of starch gels in dehydrated potato cubes, it seems evident from the foregoing observations and previous information *(14, 15)* that properties of the gelled starch contents in the tissues are significant in the control of textural qualities in processed potatoes.

EFFECTS OF HEATING, FREEZING, AND DRYING ON STRUCTURE

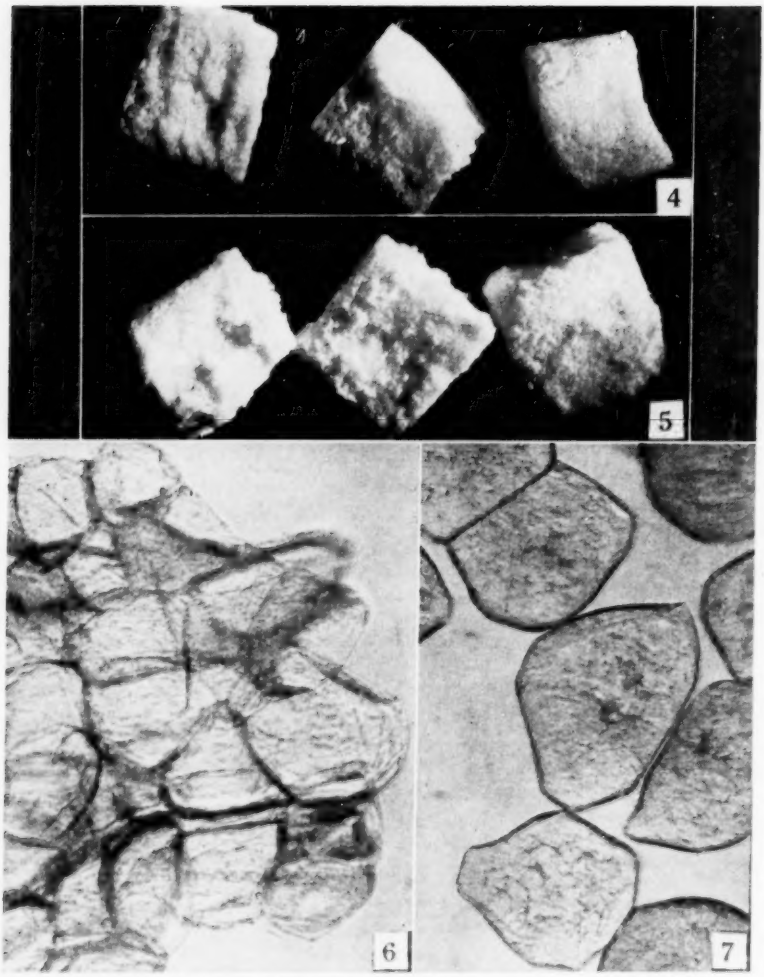
Porous dehydrated potatoes. In more recent processing investigations at this Laboratory the effects of freezing and thawing on precooked potatoes have been studied with particular reference to development of a porous dehydrated product *(10)*. Samples of porous dehydrated potatoes have been examined microscopically to learn the degree of structural alterations produced by different treatments.

Both cubes and slices of potatoes which had been cooked, frozen, thawed, and dehydrated showed a characteristically porous structure (Figures 4 and 5). Fineness of porosity varied according to the freezing and thawing treatments and also with precooking methods *(10)*. Samples frozen at −4°C.(22°F.) had a slightly coarser porosity when dried than did those frozen at −25°C.(9°F.). Rapid thawing prior to drying of tissues frozen at −25°C. tended to increase the coarseness of porosity but rate of thaw did not appear to influence porosity in samples frozen at −4°C. All samples were dehydrated, after thawing, in a tunnel drier at temperatures between 60° and 70°C. (140° and 150°F.) for about 2½ hours.

Freezing out of water from the gelled starch contents of the tissues after cooking apparently prevents tissue shrinkage and development of glassy, translucent texture upon dehydration. Consequently, the tissues dry in a porous form from which they are readily rehydrated for culinary use. The influence of rate of thaw upon fineness of porosity suggests that some water reabsorption by the gelled starch may aid in development of the finely grained porosity upon dehydration *(9, 10)*.

Microscopic examination of the porous dehydrated potatoes revealed the spongy network to be composed of fine, interconnecting strands of intact and only slightly shrunken cells. Local areas in which large strands occurred in some of the samples of coarse porosity contained more severely shrunken cells which were comparable with those of the translucent dehydrated cubes *(13)*. Voids in these porous potato products apparently resulted from the combined effects of heat-expanded intercellular gases and weakening of the middle lamellae between cell walls by the precooking treatment.

Porous dehydrated cubes prepared from both Russet Burbank and White Rose tubers provided interesting comparisons with effects of boiling and steaming on these varieties *(14, 15)*. When the porous cubes were rehydrated in cold water for 30 minutes or longer, cell separation on the microslide was not readily obtained. Bits of tissue showing partial cell separation in cold water were composed of cells considerably less shrunken and more angular in structure than were those separated by rehydration in hot water (Figures 6 and 7). This difference between samples rehydrated in cold and hot water was essentially the same with White Rose and Russet Burbank varieties. Moreover, no broken cells were observed (except for those obviously caused by cubing) in the rehydrated porous cubes prepared from either variety.



Figures 4 and 5. Porous dehydrated potato cubes from White Rose tubers. Figure 4—Steam cooked prior to freezing and dehydration. 2 ×. Figure 5—Boiled prior to freezing and dehydration. Center cubes in both Figure 4 and Figure 5 broken to show internal texture which, in Figure 5, is slightly more loose and granular than in Figure 4. 2 ×. Figures 6 and 7—Rehydrated cells from porous dehydrated potato cubes. 100 ×. Figure 6—Rehydration by soaking in cold water one hour. Note angular appearance of unseparated cells. Figure 7—Rehydration by soaking in hot water ten minutes. Note cell separation and slight distension of cell walls.

It should be pointed out that the precook used in preparing these porous cubes was not carried out to the point of ready cell separation. Consequently, none of the cells was sufficiently swollen to become ruptured before the cooked cubes were frozen. The lack of severe swelling and cell rupturing upon reconstitution of the dried cubes in hot water suggests that the freezing, thawing, and drying processes had altered the

water-reabsorbing capacity of the gelled starch contents. Further suggestion of starch alteration was shown by iodine staining of the rehydrated samples. Amyloid blue coloring of the soluble starch in extra-cellular solutions of rehydrated cubes of White Rose was considerably less than that observed with boiled and steamed slices *(15)*. None was observed with the rehydrated Russet Burbank samples.

Dehydrated potato granules and powders. Considerable investigation has been conducted at this Laboratory on development of potato granules *(11, 12)*. Consequently various potato granule and powder preparations have been examined microscopically.

Powders which had been prepared by grinding piece-dried potatoes contained large numbers of broken cells. Upon rehydration of these, the gelled starch liberated from the tissue fragments usually caused sticky or gummy textural qualities. On the other hand, potato granules, prepared by precooking to the point of ready cell separation with whipping, consisted largely of intact cells and cell clusters (Figures 8 to 11). In the preparation of some of these products, dehydration was aided by adding back previously dehydrated potato powder—usually to the freshly cooked product at time of whipping or stirring to separate the cells. Various modifications of this basic method of preparation are used in commercial practice.

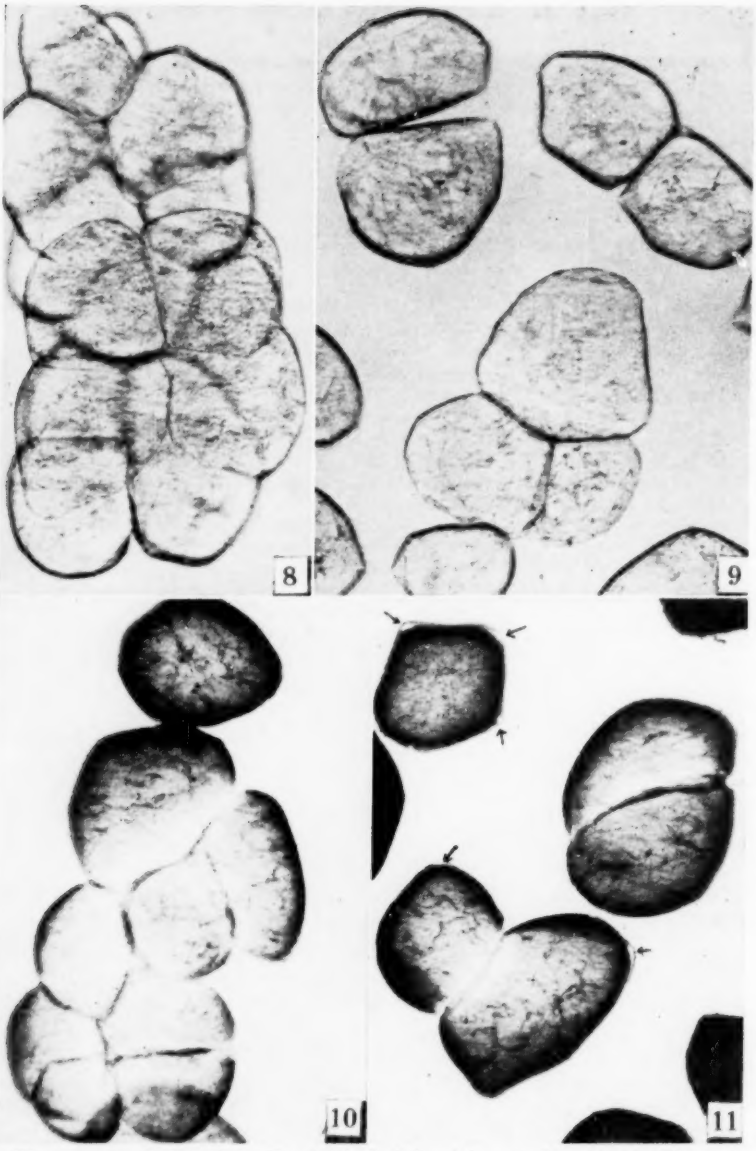
Microscopic examinations of rehydrated potato granules showed that the separated cells were not excessively swollen and, in some instances, the reswelling of the gelled starch did not completely fill the angles between adjacent cell-wall facets (Figure 11). In the dry state these separated cells were not severely shrunken and appeared to be slightly globular and wrinkled. These observations suggest that the effect of the add-back powder during preparation provides a slight initial drying. The gelled starch contents of the cooked tissues thus become more viscous so that water is less drastically removed during hot air-blast drying and the separated cells then do not undergo excessive shrinkage. Whatever the physical mechanisms of tissue change, the effect of this form of processing upon both structure and final textural quality very well illlustrate manipulative control of the physical characteristics of the gelled starch in cooked potatoes.

DISCUSSION

Although other forms of processed potatoes have not been investigated, the observations in this present survey on mechanisms of histological change may serve to explain the basic nature of some of the quality control problems encountered. In addition to control of texture, other problems of quality such as discoloration during processing or in storage, flavor retention, suitability of variety, and diversity of use demand continued fundamental investigation. Basic histological information which might aid in the elucidation of many of these problems is conspicuously meager.

The roles of cell separation, cell rupturing, and other tissue changes as influenced by gelation and swelling of starch granules have been previously recognized as important factors involving texture. Since most of the literature related to these structural changes in processed potatoes already has been reviewed *(13, 14, 15)*, only a few additional points of interest will be considered.

As stated by Campbell and coworkers *(3)*, Freeman *(5, 6)* correctly associated cell separation with mealiness and porosity in dried baked potatoes. Freeman further pointed out that nonmealy baked tubers dried into a horny, translucent mass. These observations were considered important to potato dehydration *(3)*. The localized porosity ("popcorn" effect) was characteristic of dehydrated cubes from mealy tubers but the longer blanching periods (which caused porosity and fracturing) had been considered necessary for adequate enzyme inactivation as determined by the tests then used.



Figures 8-11. Rehydrated cells of dehydrated potato granules. 100 ×. Figure 8—Cells in clumped, unseparated condition; unstained. Figure 9—Separated cells, unstained, and showing only average distension of cell walls. Particles in dehydrated granules range from those containing several cells (as in Figure 8) to single, or two or three joined cells (as in Figure 9). Figure 10—Rehydrated cells replicate to those of Figure 8 but stained lightly with iodine; note reticulation of gelled starch contents. Figure 11—Rehydrated cells replicate to those of Figure 9 but stained lightly with iodine. Note reticulation of gelled starch contents. Arrows indicate regions in which rehydrated gelled starch did not swell sufficiently to completely fill cells, leaving small spaces in the angles formed by the cell walls.

In recent years much information on improved potato products has been published *(8, 9, 10, 11, 12)*. Products in a porous form, or as granules or powders, represent advances in processing technology. Advantages of dehydrated potatoes in a porous form, or as granules or powders composed of intact cells, are nearly self-evident; they are structurally suited to rapid rehydration and culinary use. Products in the brittle, translucent form are more suitable for use in soups and stews in that they retain their identity. In each instance, the texture of the reconstituted product depends upon the physical properties of the gelled starch in the cells, including its moisture reabsorbing capacity.

Woodruff and Hayden *(16)* and Farrow and Swan *(4)* found that the moisture reabsorbing capacity of starch gels was altered by heating or by freezing. This has been the basic approach to commercial development of a number of new starch products. Longrée *(7)* also demonstrated relationship between freezing and properties of the gelled starch in cooked, frozen potatoes. The cells of cooked, frozen, and thawed potatoes remained wrinkled and smaller than those of freshly cooked tubers. "Cottoniness" of texture, as described by Longrée, can be considered as an extreme form of mealiness associated with the more shrunken cells in which the gelled starch has lost some of its moisture-reabsorbing capacity.

The presence of ruptured cells in reconstituted potato granules or powders necessarily does not indicate a relationship to the moisture reabsorption of the gelled starch in these cells. Such rupturing may be the result of cell wall abrasion when the dried product is sieved, or of other mechanical cause. Cell rupturing is considered undesirable, in any event, because the gelled starch freed from the broken cells may cause poor culinary quality. Consequently, microscopic counting of ruptured cells in the rehydrated product has been regarded as a possible approach in laboratory evaluation.

There are, however, many difficulties inherent in the microscopic approach which may affect the validity of the counts made *(9)*. It is necessary to mount the sample in water on a microslide and under a cover glass for microscopic observation. Faulty technique in preparing the sample may result in cell rupturing due to pressure on the cover glass *(9)*. Increased pressure of the cover glass also occurs with evaporation from under the edges of the cover glass. In addition, swelling of the gelled starch may continue for some time and cause more cell rupturing during observation, as described in the first paper of this series *(14)*.

Sources of error due to pressure of the cover glass can be eliminated by mounting the sample in a Rafter counting chamber. This slide has a rectangular chamber of 1 mm. depth and 1 ml. capacity over which the cover glass is placed. Since the average size of a starch storage cell in a potato is about 300 μ, the cover glass is held sufficiently above the cells in the Rafter slide to avoid pressure and possible cell rupturing. In situations in which continued cell swelling occurs, it may be necessary to determine the time at which the swelling and cell rupturing has ceased before making counts. It is questionable, however, whether such a procedure would provide results which truly represent reconstitution conditions and culinary qualities.

Because different forms of dehydrated potatoes obviously are suited to only limited culinary or institutional purposes for each product, the development of more versatile products seemingly is desirable. Since starch is important to texture in most processed potato products, it logically follows that more complete information on the alterations of starch, *in situ*, by various processing methods, is desirable for a better understanding of textural qualities and development of new products.

SUMMARY

Histological observations on various forms of dehydrated potato products have further demonstrated relationship between textural qualities and the physical properties of the gelled starch contents of the cells. Pretreatments of heating, freezing, and thawing alter the moisture-reabsorbing capacity of the starch gels. This effect is reflected in the cell size (reswelling) and texture of the reconstituted product; it also influences degree of porosity in porous, dried potatoes.

Continued investigation of the properties of gelled starch, *in situ*, and of other histological changes caused by processing treatments would be pertinent to the development of new products.

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